

CONFORMATIONALLY FLEXIBLE CATIONIC CONJUGATED POLYMERS

Inventors:

Guillermo C. Bazan
Santa Barbara, California
Citizenship: United States of America

Bin Liu
Goleta, California
Citizenship: Peoples Republic of China

Assignee:

The Regents of the University of California
1111 Franklin Street, Twelfth Floor
Oakland, CA 94607-5200

Prepared By:
David W. Maher
Bingham McCutchen LLP
Three Embarcadero Center, Suite 1800
San Francisco, California 94111
(650) 849-4908

CONFORMATIONALLY FLEXIBLE CATIONIC CONJUGATED POLYMERS

5

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

Work leading to this invention was performed under grant number GM62958-01 from the National Institutes of Health, grant number DMR-0097611 from the National Science Foundation and grant number N00014-98-1-0759 from the Office of Naval
10 Research. The U.S. Government may have limited rights in this invention.

TECHNICAL FIELD

This invention relates to methods, articles and compositions involving cationic conjugated polymers ("CCPs").

15

BACKGROUND OF THE INVENTION

Conjugated polymers (CPs) are efficient light-gathering molecules with properties desirable for a variety of applications. Conjugated polymers can serve as light harvesting materials and signal transducers in fluorescent biosensor applications.^{1,2} These molecules
20 can detect, transduce and/or amplify chemical, biological or physical information into optical and/or electrical signals.^{3,4} CPs can provide the advantage of collective response relative to non-interacting small molecules.^{5,6} This collective response influences optoelectronic properties, such as Förster resonance energy transfer (FRET), electrical conductivity and fluorescence efficiency, properties which can be used to report, or
25 "transduce," target analyte presence.⁶

Water solubility of CPs, a prerequisite for interrogating biological substrates, is usually achieved by charged groups attached to the CP backbone.⁷ To date, however, most of the available ionic conjugated polymers are polyanions containing sulfonate or carboxylate functionalities.

5 Conjugated polymers frequently take the form of rigid-rod structures which have limited flexibility and consequently have a limited ability to adapt to particular three dimensional shapes, thus limiting their ability to conform to the shape of biologically-derived molecules. For example, proteins and nucleic acids, although also polymeric, do not typically form extended-rod structures but rather fold into higher-order three-
10 dimensional shapes to which CPs cannot typically conform.

 All the currently available cationic water-soluble conjugated polymers have generally linear “rigid-rod” polymer backbones and therefore experience a limited twist angle between monomer units along the polymer main chain. A consequence of this torsional restriction is that the polymer has a “rigid rod” structure with limited
15 conformations and ability to adapt to the secondary structures of bio-molecules. Additionally, when cationic conjugated polymers are used as light-harvesting molecules, they can deleteriously exhibit fluorescence self-quenching when they cluster near negatively charged biomolecules.

 There is a need in the art for novel CCPs, for methods of making and using them,
20 and for compositions and articles of manufacture comprising such compounds.

SUMMARY OF THE INVENTION

 Methods, compositions and articles of manufacture involving cationic conjugated conformationally flexible polymers are provided. A method for the synthesis of cationic
25 water-soluble polymers with linkages along the polymer main chain structure which disrupt the ability of the polymers to form extended-rod structures is provided. Such polymers may serve in the fabrication of novel optoelectronic devices and in the development of highly efficient biosensors. The invention further relates to the application of these polymers in assay methods.

Advantageously, the methods allow for modification of the shape of the polymers and can provide the ability to control their emission properties. Cationic conjugated polymers are provided that can better adapt to the secondary structure of biological substrates, exhibit reduced chain packing and/or exhibit reduced self-quenching in solution.

5 The polymers provided may emit at different wavelengths which may be useful in multiplexed homogenous or surface-bound biosensors.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the absorption spectra of cationic water-soluble polymers ($M_{100}P_0^+$, $M_{90}P_{10}^+$, $M_{75}P_{25}^+$, $M_{50}P_{50}^+$, $M_{25}P_{75}^+$, $M_0P_{100}^+$) in water.

10

Figure 2 presents the emission spectra of cationic water-soluble polymers ($M_{100}P_0^+$, $M_{90}P_{10}^+$, $M_{75}P_{25}^+$, $M_{50}P_{50}^+$, $M_{25}P_{75}^+$, $M_0P_{100}^+$) in water.

Figure 3 depicts the emission spectra of $M_{50}P_{50}^+$ and $M_0P_{100}^+$, and the absorption spectrum of ds-DNA-C* (C* = fluorescein) in water. The excitation wavelength is 360 nm for $M_{50}P_{50}^+$, and 380 nm for $M_0P_{100}^+$.

15

Figure 4 depicts the emission spectra from solutions containing $M_{100}P_0^+$ (345 nm), $M_{90}P_{10}^+$ (345 nm), $M_{75}P_{25}^+$ (353 nm), $M_{50}P_{50}^+$ (360 nm), $M_{25}P_{75}^+$ (375 nm), $M_0P_{100}^+$ (380 nm) and ds-DNA-C* in 50 mmol phosphate buffer (pH = 8.0) ([ds-DNA-C*] = 2.0 E-8 M, [polymer repeat unit] = 5.0 E-7 M). The excitation wavelength is in parenthesis.

Figure 5 presents a schematic representation for the interaction of cationic water soluble conjugated polymers with different shape of polymers with ds-DNA-C*.

20

Figure 6. Comparison of the C* intensity for $M_{50}P_{50}^+$ /ds-DNA-C* and $M_0P_{100}^+$ /ds-DNA-C* in 50 mmol phosphate buffer (pH = 8.0) with polymer concentration varying from 1.0 E-7 M to 1.0 E-6 M at a ds-DNA-C* concentration of 2.0 E-8 M. The excitation wavelength is 363 nm for both $M_{50}P_{50}^+$ and $M_0P_{100}^+$.

25

Figure 7. Schematic illustration of a strand specific DNA assay based on a cationic water-soluble conjugated polymer (in black) in conjunction with a specific ss-DNA-C* optical probe (in red) to detect a complementary ss-DNA sequence (in blue). The non-complementary ss-DNA is shown in green.

Figure 8. Emission spectra from solutions containing $M_{50}P_{50}^{+}$, complimentary ds-DNA-C* and non-complimentary ss-DNA-C*/DNA and $M_0P_{100}^{+}$, complimentary ds-DNA-C* and non-complimentary ss-DNA-C*/DNA in 50 mmol phosphate buffer (pH = 8.0, [ds-DNA-C*] = [ss-DNA-C*] = 2.0 E-8 M, [polymer repeat unit] = 4.2 E-7 M). The excitation wavelength is 360 nm for $M_{50}P_{50}^{+}$ and 380 nm for $M_0P_{100}^{+}$. For each polymer, the polymer emission spectra were normalized for comparison.

Figure 9. Emission spectra from solutions containing $M_{50}P_{50}^{+}$, complimentary ds-PNA-C*/DNA (PNA = peptide nucleic acid) and non-complimentary ss-PNA-C*/DNA in 50 mmol phosphate buffer (pH = 6.0, [ds-PNA-C*/DNA] = [ss-PNA-C*/DNA] = 2.0 E-8 M, [$M_{50}P_{50}^{+}$ repeat unit] = 2.5 E-7 M). The excitation wavelength is 360 nm. The spectra were normalized with respect to the polymer emission.

Figure 10. Comparison of the intensity of EB (EB = Ethidium bromide) emission for polymer/ds-DNA/EB in 50 mmol phosphate buffer (pH = 7.4) with [ds-DNA] = 1.0 E-8 M, [Polymer RU] = 2.0 E-7 M, [EB] = 1.1 E-6 M. Emission intensity was normalized relative to the ϵ value at the excitation wavelength.

DETAILED DESCRIPTION OF THE INVENTION

The inventors have provided cationic conjugated polymers (CCPs) comprising monomers which perturb the polymer's ability to form rigid-rod structures, allowing them to form a greater range of three-dimensional structures. The monomers are aromatic molecules having attachment points to the adjacent subunits of the polymer which form an angle of greater than about 25°. The monomers may introduce a torsional twist in the conjugated polymer, thereby further disrupting the ability of the polymer to form a rigid-rod structure.

A synthetic method is also provided for producing CCPs with a range of backbone regiochemistries. Such CCPs exhibit facile energy transfer amongst polymer segments which results in similar emission properties and FRET function, despite structural differences which affect the average conjugation length. Furthermore, the flexible CCPs

can be more efficient excitation donors with respect to particular biomolecules and under certain conditions these materials can show improved performance when used in bioassays that take advantage of the optical amplification of water-soluble conjugated polymers.

5 In one aspect, a plurality of CCPs with different structures are provided, which may be in the form of a library. The CCPs may be tested for any property of interest. Particular biological targets of interest can be tested against a plurality of different CCPs comprising such subunits to find particular CCPs with properties desirable for use with the target. For example, the CCPs may be tested for binding to the target and/or for energy transfer to the target, for increased fluorescent efficiency, for decreased self-quenching, increased Stoke's
10 shift, and for emission wavelength.

Also provided are methods of use of the CCPs in bioassays for target biomolecules. The CCPs may be provided in solution and/or kit form, which may be adapted for performing specific assay methods. Sensing complexes and solutions comprising a CCP are also provided, as are detection complexes comprising a CCP, a sensor and a signaling
15 chromophore. Articles of manufacture comprising the CCP are also provided. Other aspects of the invention are discussed further herein.

In one aspect, a method is provided comprising contacting a sample suspected of comprising a target with a solution comprising a flexible CCP, a sensor biomolecule, and a luminescent signaling chromophore. The CCP and the signaling chromophore (C^*) are
20 chosen so that the absorption bands of the two species have minimal overlap and so that the luminescent emission spectra of the two species are at different wavelengths. A detectable change in the emission of light with wavelength characteristic of the signaling chromophore indicates the presence of the target, which binds to the sensor and forms a detection complex, in the sample. By using multiple sensor biomolecules, each with a
25 different binding specificity, multiple targets can be independently detected. An additional component such as a dye may be introduced to improve selectivity by further transferring energy from the signaling chromophore to the dye.

In addition to the described method, the invention provides a predominantly aqueous solution comprising a CCP, a “sensor biomolecule” and a signaling chromophore.

As demonstrated in the Examples, the optical amplification provided by a flexible CCP can be used to detect polynucleotide hybridization to a sensor polynucleotide. The amplification can be enhanced by using higher molecular weight water CCPs. The invention can be provided in a homogeneous format that utilizes the ease of fluorescence detection methods. The methods can be used to detect amplified target polynucleotides or, because of the large signal amplification, as a stand alone assay, without need for polynucleotide amplification.

The methods of the invention can all be performed in multiplex formats. A plurality of different sensor biomolecules can be used to detect corresponding different target biomolecules in a sample through the use of different signaling chromophores conjugated to the respective sensor biomolecules. Multiplex methods are provided employing 2, 3, 4, 5, 10, 15, 20, 25, 50, 100, 200, 400 or more different sensor biomolecules which can be used simultaneously to assay for corresponding different target biomolecules.

The methods can be performed on a substrate, as well as in solution, although the solution format is expected to be more rapid due to diffusion issues. Thus the assay can be performed, for example, in an array format on a substrate, which can be a biosensor. This can be achieved by anchoring or otherwise incorporating an assay component onto the substrate, for example the sensor biomolecule, the CCP, or both. These substrates may be surfaces of glass, silicon, paper, plastic, or the surfaces of optoelectronic semiconductors (such as, but not confined to, indium-doped gallium nitride or polymeric polyanilines, etc.) employed as optoelectronic transducers. The location of a given sensor biomolecule may be known or determinable in an array format, and the array format may be microaddressable or nanoaddressable. In one variation, one or more samples, which may contain an amplification product, can be attached to the substrate, and the substrate can be contacted with one or more labeled sensor biomolecules and the CCP.

Before the present invention is described in further detail, it is to be understood that this invention is not limited to the particular methodology, devices, solutions or apparatuses described, as such methods, devices, solutions or apparatuses can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular
5 embodiments only, and is not intended to limit the scope of the present invention.

Use of the singular forms “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. Thus, for example, reference to “a cationic conjugated polymer” includes a plurality of cationic conjugated polymers, reference to “a subunit” includes a plurality of such subunits, reference to “a sensor” includes a plurality of sensors,
10 and the like. Additionally, use of specific plural references, such as “two,” “three,” etc., read on larger numbers of the same subject less the context clearly dictates otherwise.

Terms such as “connected,” “attached,” and “linked” are used interchangeably herein and encompass direct as well as indirect connection, attachment, linkage or conjugation unless the context clearly dictates otherwise. Where a range of values is
15 recited, it is to be understood that each intervening integer value, and each fraction thereof, between the recited upper and lower limits of that range is also specifically disclosed, along with each subrange between such values. The upper and lower limits of any range can independently be included in or excluded from the range, and each range where either, neither or both limits are included is also encompassed within the invention. Where a
20 value being discussed has inherent limits, for example where a component can be present at a concentration of from 0 to 100%, or where the pH of an aqueous solution can range from 1 to 14, those inherent limits are specifically disclosed. Where a value is explicitly recited, it is to be understood that values which are about the same quantity or amount as the recited value are also within the scope of the invention, as are ranges based thereon. Where a
25 combination is disclosed, each subcombination of the elements of that combination is also specifically disclosed and is within the scope of the invention. Conversely, where different elements or groups of elements are disclosed, combinations thereof are also disclosed. Where any element of an invention is disclosed as having a plurality of alternatives, examples of that invention in which each alternative is excluded singly or in any

combination with the other alternatives are also hereby disclosed; more than one element of an invention can have such exclusions, and all combinations of elements having such exclusions are hereby disclosed.

5 Unless defined otherwise or the context clearly dictates otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, the preferred methods and materials are now described.

10 All publications mentioned herein are hereby incorporated by reference for the purpose of disclosing and describing the particular materials and methodologies for which the reference was cited. The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

15

DEFINITIONS

In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

20 "Alkyl" refers to a branched, unbranched or cyclic saturated hydrocarbon group of 1 to 24 carbon atoms optionally substituted at one or more positions, and includes polycyclic compounds. Examples of alkyl groups include optionally substituted methyl, ethyl, n-propyl, isopropyl, n-butyl, s-butyl, t-butyl, n-pentyl, isopentyl, neopentyl, n-hexyl, n-heptyl, n-octyl, n-decyl, hexyloctyl, tetradecyl, hexadecyl, eicosyl, tetracosyl and the like, as well as cycloalkyl groups such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, adamantyl, and norbornyl. The term "lower alkyl" refers to an
25 alkyl group of 1 to 6 carbon atoms, preferably 1 to 4 carbon atoms. Exemplary substituents on substituted alkyl groups include hydroxyl, cyano, alkoxy, =O, =S, -NO₂, halogen, haloalkyl, heteroalkyl, carboxyalkyl, amine, amide, thioether and -SH.

“Alkoxy” refers to an “-Oalkyl” group, where alkyl is as defined above. A “lower alkoxy” group intends an alkoxy group containing one to six, more preferably one to four, carbon atoms.

“Alkenyl” refers to a branched, unbranched or cyclic hydrocarbon group of 2 to 24
5 carbon atoms containing at least one carbon-carbon double bond optionally substituted at one or more positions. Examples of alkenyl groups include ethenyl, 1-propenyl, 2-propenyl (allyl), 1-methylvinyl, cyclopropenyl, 1-butenyl, 2-butenyl, isobutenyl, 1,4-butadienyl, cyclobutenyl, 1-methylbut-2-enyl, 2-methylbut-2-en-4-yl, prenyl, pent-1-enyl, pent-3-enyl, 1,1-dimethylallyl, cyclopentenyl, hex-2-enyl, 1-methyl-1-ethylallyl,
10 cyclohexenyl, heptenyl, cycloheptenyl, octenyl, cyclooctenyl, decenyl, tetradecenyl, hexadecenyl, eicosenyl, tetracosenyl and the like. Preferred alkenyl groups herein contain 2 to 12 carbon atoms. The term “lower alkenyl” intends an alkenyl group of 2 to 6 carbon atoms, preferably 2 to 4 carbon atoms. The term “cycloalkenyl” intends a cyclic alkenyl group of 3 to 8, preferably 5 or 6, carbon atoms. Exemplary substituents on substituted
15 alkenyl groups include hydroxyl, cyano, alkoxy, =O, =S, -NO₂, halogen, haloalkyl, heteroalkyl, amine, thioether and -SH.

“Alkenyloxy” refers to an “-Oalkenyl” group, wherein alkenyl is as defined above.

“Alkylaryl” refers to an alkyl group that is covalently joined to an aryl group. Preferably, the alkyl is a lower alkyl. Exemplary alkylaryl groups include benzyl,
20 phenethyl, phenopropyl, 1-benzylethyl, phenobutyl, 2-benzylpropyl and the like.

“Alkylaryloxy” refers to an “-Oalkylaryl” group, where alkylaryl is as defined above.

“Alkynyl” refers to a branched or unbranched hydrocarbon group of 2 to 24 carbon atoms containing at least one -C≡C- triple bond, optionally substituted at one or more
25 positions. Examples of alkynyl groups include ethynyl, n-propynyl, isopropynyl, propargyl, but-2-ynyl, 3-methylbut-1-ynyl, octynyl, decynyl and the like. Preferred alkynyl groups herein contain 2 to 12 carbon atoms. The term “lower alkynyl” intends an alkynyl group of 2 to 6, preferably 2 to 4, carbon atoms, and one -C≡C- triple bond. Exemplary substituents on substituted alkynyl groups include hydroxyl, cyano, alkoxy, =O, =S, -NO₂,
30 halogen, haloalkyl, heteroalkyl, amine, thioether and -SH.

“Amide” refers to $-C(O)NR'R''$, where R' and R'' are independently selected from hydrogen, alkyl, aryl, and alkylaryl.

“Amine” refers to an $-N(R')R''$ group, where R' and R'' are independently selected from hydrogen, alkyl, aryl, and alkylaryl.

5 “Aryl” refers to an aromatic group that has at least one ring having a conjugated pi electron system and includes carbocyclic, heterocyclic, bridged and/or polycyclic aryl groups, and can be optionally substituted at one or more positions. Typical aryl groups contain 1 to 5 aromatic rings, which may be fused and/or linked. Exemplary aryl groups include phenyl, furanyl, azolyl, thiofuranyl, pyridyl, pyrimidyl, pyrazinyl, triazinyl,
10 biphenyl, indenyl, benzofuranyl, indolyl, naphthyl, quinolinyl, isoquinolinyl, quinazolinyl, pyridopyridinyl, pyrrolopyridinyl, purinyl, tetralinyl and the like. Exemplary substituents on optionally substituted aryl groups include alkyl, alkoxy, alkylcarboxy, alkenyl, alkenyloxy, alkenylcarboxy, aryl, aryloxy, alkylaryl, alkylaryloxy, fused saturated or unsaturated optionally substituted rings, halogen, haloalkyl, heteroalkyl, $-S(O)R$, sulfonyl, -
15 SO_3R , $-SR$, $-NO_2$, $-NRR'$, $-OH$, $-CN$, $-C(O)R$, $-OC(O)R$, $-NHC(O)R$, $-(CH_2)_nCO_2R$ or $-(CH_2)_nCONRR'$ where n is 0-4, and wherein R and R' are independently H, alkyl, aryl or alkylaryl.

“Aryloxy” refers to an “-Oaryl” group, where aryl is as defined above.

20 “Carbocyclic” refers to an optionally substituted compound containing at least one ring and wherein all ring atoms are carbon, and can be saturated or unsaturated.

“Carbocyclic aryl” refers to an optionally substituted aryl group wherein the ring atoms are carbon.

“Halo” or “halogen” refers to fluoro, chloro, bromo or iodo. “Halide” refers to the anionic form of the halogens.

25 “Haloalkyl” refers to an alkyl group substituted at one or more positions with a halogen, and includes alkyl groups substituted with only one type of halogen atom as well as alkyl groups substituted with a mixture of different types of halogen atoms. Exemplary haloalkyl groups include trihalomethyl groups, for example trifluoromethyl.

30 “Heteroalkyl” refers to an alkyl group wherein one or more carbon atoms and associated hydrogen atom(s) are replaced by an optionally substituted heteroatom, and

includes alkyl groups substituted with only one type of heteroatom as well as alkyl groups substituted with a mixture of different types of heteroatoms. Heteroatoms include oxygen, sulfur, and nitrogen. As used herein, nitrogen heteroatoms and sulfur heteroatoms include any oxidized form of nitrogen and sulfur, and any form of nitrogen having four covalent
5 bonds including protonated forms. An optionally substituted heteroatom refers to replacement of one or more hydrogens attached to a nitrogen atom with alkyl, aryl, alkylaryl or hydroxyl.

“Heterocyclic” refers to a compound containing at least one saturated or unsaturated ring having at least one heteroatom and optionally substituted at one or more positions.

10 Typical heterocyclic groups contain 1 to 5 rings, which may be fused and/or linked, where the rings each contain five or six atoms. Examples of heterocyclic groups include piperidinyl, morpholinyl and pyrrolidinyl. Exemplary substituents for optionally substituted heterocyclic groups are as for alkyl and aryl at ring carbons and as for heteroalkyl at heteroatoms.

15 “Heterocyclic aryl” refers to an aryl group having at least 1 heteroatom in at least one aromatic ring. Exemplary heterocyclic aryl groups include furanyl, thienyl, pyridyl, pyridazinyl, pyrrolyl, N-lower alkyl-pyrrolo, pyrimidyl, pyrazinyl, triazinyl, tetrazinyl, triazolyl, tetrazolyl, imidazolyl, bipyridyl, tripyridyl, tetrapyridyl, phenazinyl, phenanthrolinyl, purinyl and the like.

20 “Hydrocarbyl” refers to hydrocarbyl substituents containing 1 to about 20 carbon atoms, including branched, unbranched and cyclic species as well as saturated and unsaturated species, for example alkyl groups, alkylidenyl groups, alkenyl groups, alkylaryl groups, aryl groups, and the like. The term “lower hydrocarbyl” intends a hydrocarbyl group of one to six carbon atoms, preferably one to four carbon atoms.

25 A “substituent” refers to a group that replaces one or more hydrogens attached to a carbon or nitrogen. Exemplary substituents include alkyl, alkylidenyl, alkylcarboxy, alkoxy, alkenyl, alkenylcarboxy, alkenyloxy, aryl, aryloxy, alkylaryl, alkylaryloxy, -OH, amide, carboxamide, carboxy, sulfonyl, =O, =S, -NO₂, halogen, haloalkyl, fused saturated or unsaturated optionally substituted rings, -S(O)R, -SO₃R, -SR, -NRR', -OH, -CN, -
30 C(O)R, -OC(O)R, -NHC(O)R, -(CH₂)_nCO₂R or -(CH₂)_nCONRR' where n is 0-4, and

wherein R and R' are independently H, alkyl, aryl or alkylaryl. Substituents also include replacement of a carbon atom and one or more associated hydrogen atoms with an optionally substituted heteroatom.

“Sulfonyl” refers to -S(O)₂R, where R is alkyl, aryl, -C(CN)=C-aryl, -CH₂CN, alkylaryl, or amine.

“Thioamide” refers to -C(S)NR'R'', where R' and R'' are independently selected from hydrogen, alkyl, aryl, and alkylaryl.

“Thioether” refers to -SR, where R is alkyl, aryl, or alkylaryl.

The term “antibody” as used herein includes antibodies obtained from both polyclonal and monoclonal preparations, as well as: hybrid (chimeric) antibody molecules (see, for example, Winter et al. (1991) Nature 349:293-299; and U.S. Patent No. 4,816,567); F(ab')₂ and F(ab) fragments; Fv molecules (noncovalent heterodimers, see, for example, Inbar et al. (1972) Proc Natl Acad Sci USA 69:2659-2662; and Ehrlich et al. (1980) Biochem 19:4091-4096); single-chain Fv molecules (sFv) (see, for example, Huston et al. (1988) Proc Natl Acad Sci USA 85:5879-5883); dimeric and trimeric antibody fragment constructs; minibodies (see, e.g., Pack et al. (1992) Biochem 31:1579-1584; Cumber et al. (1992) J Immunology 149B:120-126); humanized antibody molecules (see, for example, Riechmann et al. (1988) Nature 332:323-327; Verhoeyan et al. (1988) Science 239:1534-1536; and U.K. Patent Publication No. GB 2,276,169, published 21 September 1994); and, any functional fragments obtained from such molecules, wherein such fragments retain specific-binding properties of the parent antibody molecule.

As used herein, the term “monoclonal antibody” refers to an antibody composition having a homogeneous antibody population. The term is not limited regarding the species or source of the antibody, nor is it intended to be limited by the manner in which it is made. Thus, the term encompasses antibodies obtained from murine hybridomas, as well as human monoclonal antibodies obtained using human hybridomas or from murine hybridomas made from mice expression human immunoglobulin chain genes or portions

thereof. See, e.g., Cote, et al. Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, 1985, p. 77.

The terms “polynucleotide,” “oligonucleotide,” “nucleic acid” and “nucleic acid molecule” are used interchangeably herein to refer to a polymeric form of nucleotides of any length, and may comprise ribonucleotides, deoxyribonucleotides, analogs thereof, or mixtures thereof. These terms refer only to the primary structure of the molecule. Thus, the terms includes triple-, double- and single-stranded deoxyribonucleic acid (“DNA”), as well as triple-, double- and single-stranded ribonucleic acid (“RNA”). It also includes modified, for example by alkylation, and/or by capping, and unmodified forms of the polynucleotide.

Whether modified or unmodified, when a polynucleotide is used as a sensor molecule in methods as described herein, the sensor polynucleotide can be anionic (e.g., RNA or DNA), or the sensor polynucleotide may have an uncharged backbone (e.g., PNA). The target polynucleotide can in principle be charged or uncharged, although typically it is expected to be anionic, for example RNA or DNA.

More particularly, the terms “polynucleotide,” “oligonucleotide,” “nucleic acid” and “nucleic acid molecule” include polydeoxyribonucleotides (containing 2-deoxy-D-ribose), polyribonucleotides (containing D-ribose), including tRNA, rRNA, hRNA, and mRNA, whether spliced or unspliced, any other type of polynucleotide which is an N- or C-glycoside of a purine or pyrimidine base, and other polymers containing a phosphate or other polyanionic backbone, and other synthetic sequence-specific nucleic acid polymers providing that the polymers contain nucleobases in a configuration which allows for base pairing and base stacking, such as is found in DNA and RNA. There is no intended distinction in length between the terms “polynucleotide,” “oligonucleotide,” “nucleic acid” and “nucleic acid molecule,” and these terms are used interchangeably herein. These terms refer only to the primary structure of the molecule. Thus, these terms include, for example, 3'-deoxy-2',5'-DNA, oligodeoxyribonucleotide N3' P5' phosphoramidates, 2'-O-alkyl-substituted RNA, double- and single-stranded DNA, as well as double- and single-stranded RNA, and hybrids thereof including for example hybrids between DNA and RNA, and also

include known types of modifications, for example, labels, alkylation, "caps," substitution of one or more of the nucleotides with an analog, internucleotide modifications such as, for example, those with negatively charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those containing pendant moieties, such as, for example, proteins (including enzymes (e.g. nucleases), toxins, antibodies, signal peptides, poly-L-lysine, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those containing chelates (of, e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, those with modified linkages (e.g., alpha anomeric nucleic acids, etc.), as well as unmodified forms of the polynucleotide or oligonucleotide.

10 It will be appreciated that, as used herein, the terms "nucleoside" and "nucleotide" will include those moieties which contain not only the known purine and pyrimidine bases, but also other heterocyclic bases which have been modified. Such modifications include methylated purines or pyrimidines, acylated purines or pyrimidines, or other heterocycles. Modified nucleosides or nucleotides can also include modifications on the sugar moiety, e.g., wherein one or more of the hydroxyl groups are replaced with halogen, aliphatic groups, or are functionalized as ethers, amines, or the like. The term "nucleotidic unit" is intended to encompass nucleosides and nucleotides.

 Furthermore, modifications to nucleotidic units include rearranging, appending, substituting for or otherwise altering functional groups on the purine or pyrimidine base which form hydrogen bonds to a respective complementary pyrimidine or purine. The resultant modified nucleotidic unit optionally may form a base pair with other such modified nucleotidic units but not with A, T, C, G or U. Abasic sites may be incorporated which do not prevent the function of the polynucleotide; preferably the polynucleotide does not comprise abasic sites. Some or all of the residues in the polynucleotide can optionally be modified in one or more ways.

 Standard A-T and G-C base pairs form under conditions which allow the formation of hydrogen bonds between the N3-H and C4-oxy of thymidine and the N1 and C6-NH₂, respectively, of adenosine and between the C2-oxy, N3 and C4-NH₂, of cytidine and the C2-NH₂, N'-H and C6-oxy, respectively, of guanosine. Thus, for example, guanosine (2-

amino-6-oxy-9- β -D-ribofuranosyl-purine) may be modified to form isoguanosine (2-oxy-6-amino-9- β -D-ribofuranosyl-purine). Such modification results in a nucleoside base which will no longer effectively form a standard base pair with cytosine. However, modification of cytosine (1- β -D-ribofuranosyl-2-oxy-4-amino-pyrimidine) to form isocytosine (1- β -D-ribofuranosyl-2-amino-4-oxy-pyrimidine) results in a modified nucleotide which will not effectively base pair with guanosine but will form a base pair with isoguanosine.

Isocytosine is available from Sigma Chemical Co. (St. Louis, MO); isocytidine may be prepared by the method described by Switzer et al. (1993) *Biochemistry* 32:10489-10496 and references cited therein; 2'-deoxy-5-methyl-isocytidine may be prepared by the method of Tor et al. (1993) *J. Am. Chem. Soc.* 115:4461-4467 and references cited therein; and isoguanine nucleotides may be prepared using the method described by Switzer et al. (1993), *supra*, and Mantsch et al. (1993) *Biochem.* 14:5593-5601, or by the method described in U.S. Patent No. 5,780,610 to Collins et al. Other nonnatural base pairs may be synthesized by the method described in Piccirilli et al. (1990) *Nature* 343:33-37 for the synthesis of 2,6-diaminopyrimidine and its complement (1-methylpyrazolo-[4,3]pyrimidine-5,7-(4H,6H)-dione). Other such modified nucleotidic units which form unique base pairs are known, such as those described in Leach et al. (1992) *J. Am. Chem. Soc.* 114:3675-3683 and Switzer et al., *supra*.

"Preferential binding" or "preferential hybridization" refers to the increased propensity of one biomolecule to bind to a binding partner in a sample as compared to another component of the sample.

Hybridization conditions will typically include salt concentrations of less than about 1M, more usually less than about 500 mM and preferably less than about 200 mM. In the case of hybridization between a peptide nucleic acid and a polynucleotide, the hybridization can be done in solutions containing little or no salt. Hybridization temperatures can be as low as 5°C, but are typically greater than 22°C, more typically greater than about 30°C, and preferably in excess of about 37°C. Longer fragments may require higher hybridization temperatures for specific hybridization. Other factors may affect the stringency of hybridization, including base composition and length of the

complementary strands, presence of organic solvents and extent of base mismatching, and the combination of parameters used is more important than the absolute measure of any one alone. Suitable hybridization conditions for a given assay format can be determined by one of skill in the art; nonlimiting parameters which may be adjusted include concentrations of
5 assay components, salts used and their concentration, ionic strength, temperature, buffer type and concentration, solution pH, presence and concentration of blocking reagents to decrease background binding such as repeat sequences or blocking protein solutions, detergent type(s) and concentrations, molecules such as polymers which increase the relative concentration of the polynucleotides, metal ion(s) and their concentration(s),
10 chelator(s) and their concentrations, and other conditions known in the art.

“Polypeptide” and “protein” are used interchangeably herein and include a molecular chain of amino acids linked through peptide bonds. The terms do not refer to a specific length of the product. Thus, “peptides,” “oligopeptides,” and “proteins” are included within the definition of polypeptide. The terms include polypeptides containing
15 modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations, and sulphations. In addition, protein fragments, analogs (including amino acids not encoded by the genetic code, e.g. homocysteine, ornithine, D-amino acids, and creatine), natural or artificial mutants or variants or combinations thereof, fusion proteins, and proteins comprising derivatized residues (e.g. alkylation of amine groups,
20 acetylations or others esterifications of carboxyl groups) and the like are included within the meaning of polypeptide.

“Multiplexing” herein refers to an assay or other analytical method in which multiple analytes can be assayed simultaneously.

“Optional” or “optionally” means that the subsequently described event or
25 circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs singly or multiply and instances where it does not occur at all. For example, the phrase “optionally substituted alkyl” means an alkyl moiety that may or may not be substituted and the description includes both unsubstituted, monosubstituted, and polysubstituted alkyls.

THE CONFORMATIONALLY FLEXIBLE POLYMER

The inventors have provided conformationally flexible cationic conjugated polymers (CCPs) comprising angled linkers with a substitution pattern (or regiochemistry) capable of perturbing the polymers' ability to form rigid-rod structures, allowing the CCPs to have a greater range of three-dimensional structures. The CCPs comprise at least three subunits with at least one angled linker, which may be internal and/or an end unit, and may comprise at least 4, 5, 6, 8, 10, 15, 20, 25 or more subunits. The CCPs may comprise up to about 100, 200, 300, 500, 1000, 2000, 5000, 10000, 20000, 50000 or more subunits.

The angled linker(s) are optionally substituted aromatic molecules having at least two separate bonds to other polymer components (e.g., monomers, block polymers, end groups) that are capable of forming angles relative to one another which disrupt the overall ability of the polymer to form an extended rigid-rod structure (although significant regions exhibiting such structure may remain.) The angled linkers may be bivalent or polyvalent.

The angle which the angled linkers are capable of imparting to the polymeric structure is determined as follows. Where the two bonds to other polymeric components are coplanar, the angle can be determined by extending lines representing those bonds to the point at which they intersect, and then measuring the angle between them. Where the two bonds to other polymeric components are not coplanar, the angle can be determined as follows: a first line is drawn between the two ring atoms to which the bonds attach; two bond lines are drawn, one extending from each ring atom in the direction of its respective bond to the other polymeric component to which it is joined; the angle between each bond line and the first line is fixed; and the two ring atoms are then merged into a single point by shrinking the first line to a zero length; the angle then resulting between the two bond lines is the angle the angled linker imparts to the CCP.

The angle which an angled linker is capable of imparting to the polymer is typically less than about 155°, and may be less than about 150°, less than about 145°, less than about 140°, less than about 135°, less than about 130°, less than about 125°, less than about 120°, less than about 115°, less than about 110°, less than about 105°, less than about 100°, less than about 95°, less than about 90°, less than about 85°, less than about 80°, less than about

75°, less than about 70°, less than about 65°, less than about 60°, less than about 55°, or less than about 50°. The angled linker may form an angle to its adjacent polymeric units of about 25°, 30°, 35°, 40°, 45°, 50°, 60° or more. The angled linker may introduce a torsional twist in the conjugated polymer, thereby further disrupting the ability of the polymer to form a rigid-rod structure. For angled linkers having an internally rotatable bond, such as polysubstituted biphenyl, the angled linker must be capable of imparting an angle of less than about 155° in at least one orientation.

For six-membered rings, such angles can be achieved through *ortho* or *meta* linkages to the rest of the polymer. For five-membered rings, adjacent linkages fall within this range. For eight-membered rings, linkages extending from adjacent ring atoms, from alternate ring atoms (separated by one ring atom), and from ring atoms separated by two other ring atoms fall within this range. Ring systems with more than eight ring atoms may be used. For polycyclic structures, even more diverse linkage angles can be achieved.

Exemplary linking units which meet these limitations include benzene derivatives incorporated into the polymer in the 1,2 or 1,3-positions; naphthalene derivatives incorporated into the polymer in the 1,2-, 1,3-, 1,6-, 1,7-, 1,8-positions; anthracene derivatives incorporated into the polymer in the 1,2-, 1,3-, 1,6-, 1,7-, 1,8-, and 1,9-positions; biphenyl derivatives incorporated into the polymer in the 2,3-, 2,4-, 2,6-, 3,3'-, 3,4-, 3,5-, 2,2'-, 2,3'-, 2,4'-, and 3,4'- positions; and corresponding heterocycles. The position numbers are given with reference to unsubstituted carbon-based rings, but the same relative positions of incorporation in the polymer are encompassed in substituted rings and/or heterocycles should their distribution of substituents change the ring numbering.

The CCP preferably contains at least about 0.01 mol % of the angled linker, and may contain at least about 0.02 mol %, at least about 0.05 mol %, at least about 0.1 mol %, at least about 0.2 mol %, at least about 0.5 mol %, at least about 1 mol %, at least about 2 mol %, at least about 5 mol %, at least about 10 mol %, at least about 20 mol %, or at least about 30 mol %. The CCP may contain up to 100 mol % of the angled linker, and may

contain about 99 mol % or less, about 90 mol % or less, about 80 mol % or less, about 70 mol % or less, about 60 mol % or less, about 50 mol % or less, or about 40 mol % or less.

The CCP can be a copolymer, and may be a block copolymer, a graft copolymer, or both. The angled linker may be incorporated into the CCP randomly, alternately,
5 periodically and/or in blocks. In one aspect, the angled linker can be selected from aromatic or heteroaromatic structures in which the shortest link between the linking points to the polymer involves an even number of atoms bonded to one another.

Light harvesting CCPs can efficiently transfer energy to nearby luminescent species (e.g., "signaling chromophores"). Mechanisms for energy transfer include, for example,
10 resonant energy transfer (Förster (or fluorescence) resonance energy transfer, FRET), quantum charge exchange (Dexter energy transfer) and the like. Typically, however, these energy transfer mechanisms are relatively short range; that is, close proximity of the CCP to the signaling chromophore is required for efficient energy transfer.

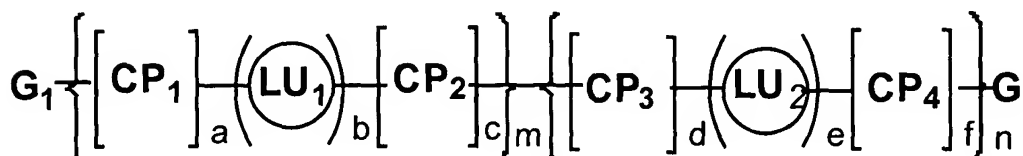
The CCPs of the present invention may desirably provide a higher quantum yield
15 and/or an increase in energy transfer to fluorescently-labeled double-stranded DNA in comparison to a copolymer of poly[9,9-bis(6'-N,N,N-trimethylammonium)hexylfluorene-co-1,4-phenylene] dibromide. Thus, the CCPs may provide at least a two-fold increase in energy transfer to labeled dsDNA, and may provide a three-fold or higher increase in energy transfer to labeled dsDNA.

20 The CCPs are polycationic, and any or all of the subunits of the polymer may comprise one or more cationic groups, including the angled linker(s). Any suitable cationic groups may be incorporated into the CCPs. Exemplary cationic groups which may be incorporated include ammonium groups, guanidinium groups, histidines, polyamines, pyridinium groups, and sulfonium groups.

25 Desirably, the CCPs described herein are soluble in aqueous solutions and other polar solvents, and preferably are soluble in water. By "water-soluble" is meant that the material exhibits solubility in a predominantly aqueous solution, which, although comprising more than 50% by volume of water, does not exclude other substances from

that solution, including without limitation buffers, blocking agents, cosolvents, salts, metal ions and detergents.

In one embodiment, an exemplary CCP is represented by Formula A:



5

Formula A

wherein:

CP₁, CP₂, CP₃, and CP₄ are optionally substituted conjugated polymer segments or oligomeric structures, and may be the same or different from one another. CP₁, CP₂, CP₃, and CP₄ may be aromatic repeat units, and may be selected from the group consisting of
 10 benzene, naphthalene, anthracene, fluorene, thiophene, furan, pyridine, and oxadiazole, each optionally substituted. Typical aromatic repeat units are shown in Table 1 below, and representative polymeric segments and oligomeric structures are shown in Table 2.

The formula contains linker units LU₁ and LU₂ which are angled linkers as described above, and can be mono- or polycyclic optionally substituted aryl groups having
 15 5 to 20 atoms. The linker units may be evenly or randomly distributed along the polymer main chain. Particularly suitable aromatic rings are those which also produce a spatial twist of the polymer main chain, preventing the conjugated polymer from forming a plane across that linker unit.

CP₁, CP₂, CP₃, CP₄, LU₁ and LU₂ are each optionally substituted at one or more
 20 positions with one or more groups selected from -R₁-A, -R₂-B, -R₃-C and -R₄-D, which may be attached through bridging functional groups -E- and -F-, with the proviso that the polymer as a whole must be substituted with a plurality of cationic groups.

R₁, R₂, R₃ and R₄ are independently selected from alkyl, alkenyl, alkoxy, alkynyl, and aryl, alkylaryl, arylalkyl, and polyalkylene oxide, each optionally substituted, which

may contain one or more heteroatoms, or may be not present. R_1 , R_2 , R_3 and R_4 can be independently selected from C_{1-22} alkyl, C_{1-22} alkoxy, C_{1-22} ester, polyalkylene oxide having from 1 to about 22 carbon atoms, cyclic crown ether having from 1 to about 22 carbon atoms, or not present. Preferably, R_1 , R_2 , R_3 and R_4 may be selected from straight or
5 branched alkyl groups having 1 to about 12 carbon atoms, or alkoxy groups with 1 to about 12 carbon atoms. It is to be understood that more than one functional group may be appended to the rings as indicated in the formulas at one or more positions.

A, B, C and D are independently selected from H, $-SiR'R''R'''$, $-N^+R'R''R'''$, a guanidinium group, histidine, a polyamine, a pyridinium group, and a sulfonium group. R' ,
10 R'' and R''' are independently selected from the group consisting of hydrogen, C_{1-12} alkyl and C_{1-12} alkoxy and C_{3-10} cycloalkyl. It is preferred that R' , R'' and R''' are lower alkyl or lower alkoxy groups.

E and F are independently selected from not present, $-O-$, $-S-$, $-C(O)-$, $-C(O)O-$, $-C(R)(R')-$, $-N(R')-$, and $-Si(R')(R'')$, wherein R' and R'' are as defined above.

15 X is O, S, Se, $-N(R')-$ or $-C(R')(R'')$, and Y and Z are independently selected from $-C(R)=$ and $-N=$, where R, R' and R'' are as defined above.

m and n are independently 0 to about 10,000, wherein $m + n > 1$. Preferably m and n are each independently 0 to about 20 and more preferably from 0 to about 10. Each repeat of m and n may be the same as or different than the other repeats thereof.

20 b and e are independently 0 to about 250, wherein $b + e > 1$.

a, c, d and f are independently 0 to about 250.

G and G1 are capping units and may be the same or different. The capping units may be activated units that allow further chemical reaction to extend the polymer chain, or may be nonactivated termination units. G and G1 can be independently selected from
25 hydrogen, optionally substituted aryl, halogen substituted aryl, boronic acid substituted aryl, and boronate radical substituted aryl.

Table 1. Typical aromatic repeat units for the construction of conjugated segments and oligomeric structures.

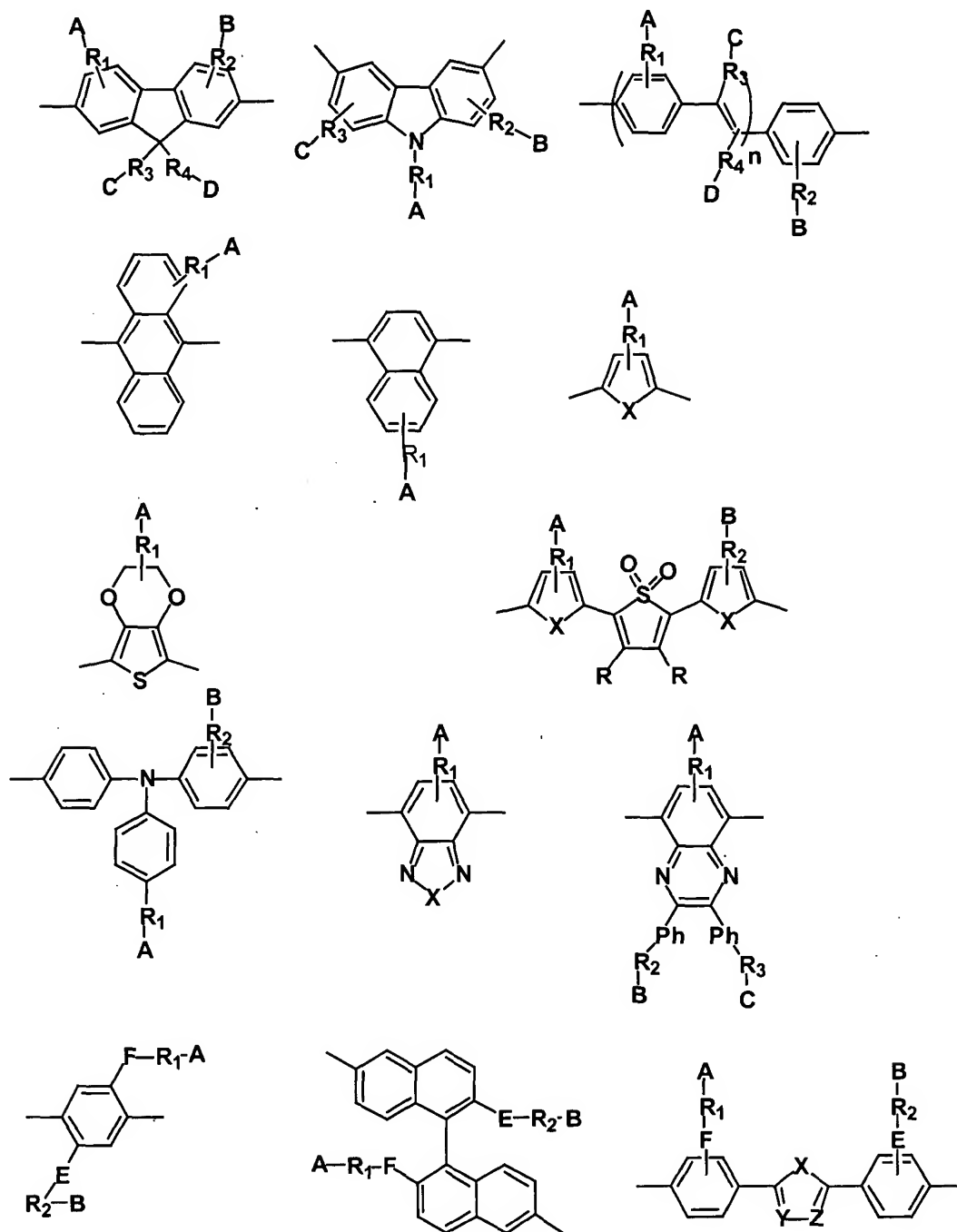
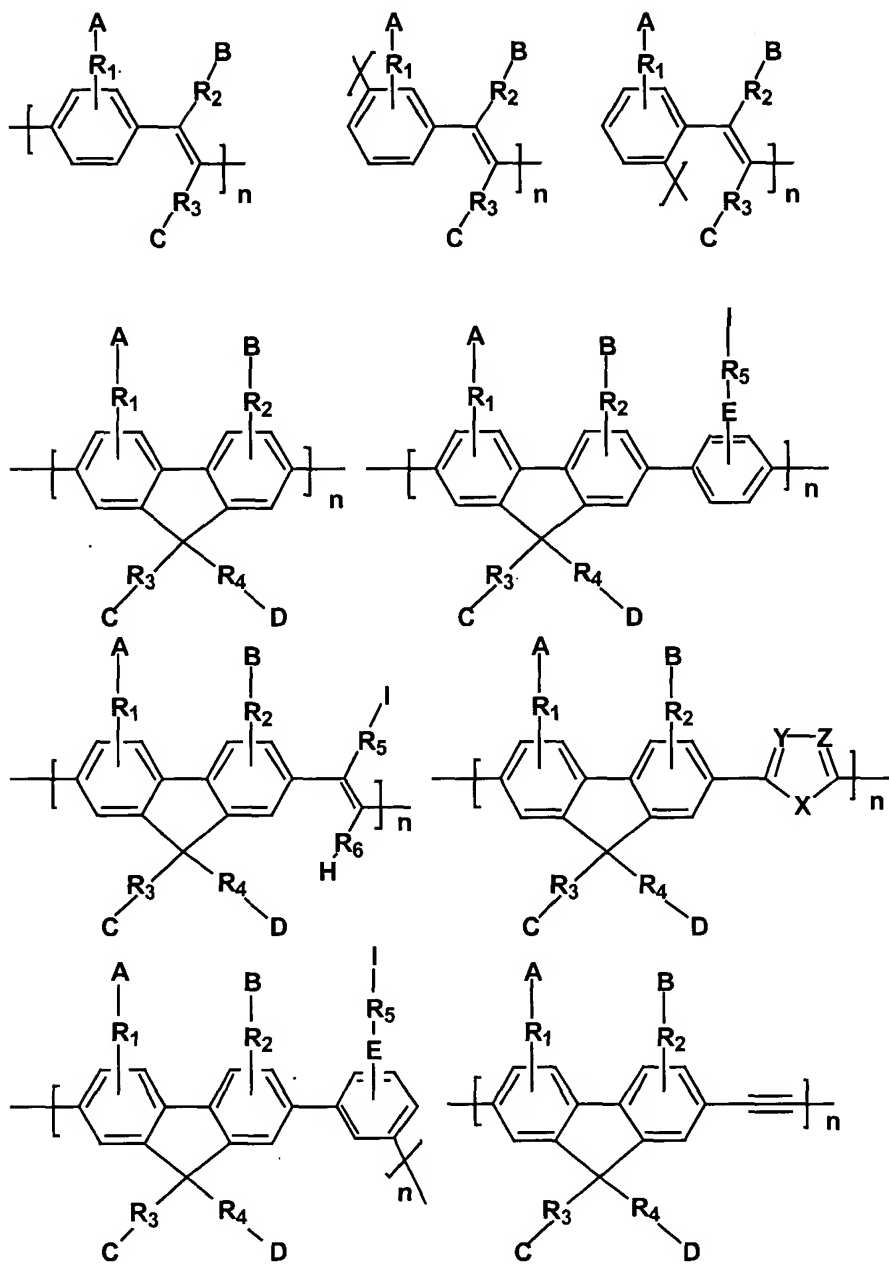
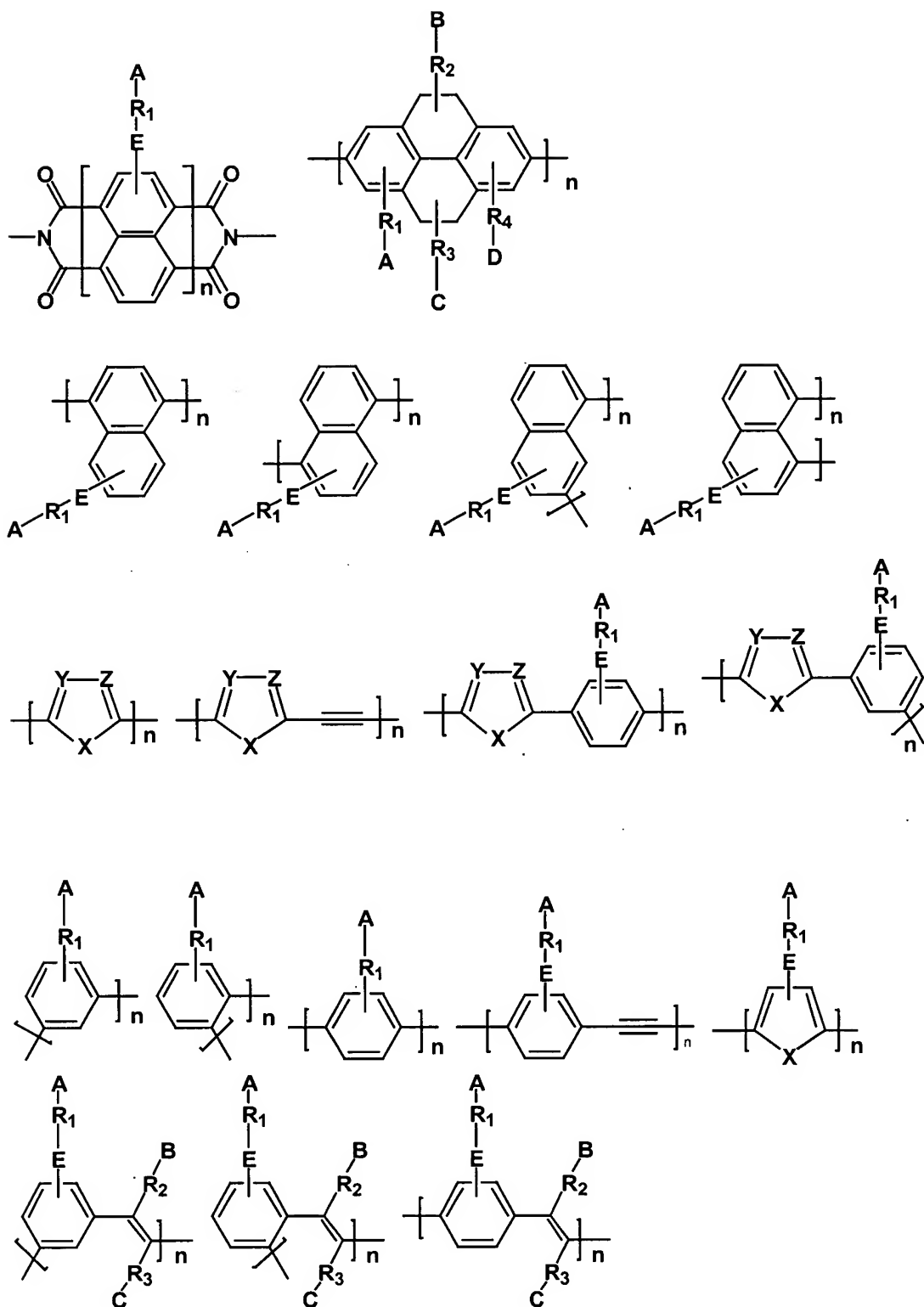
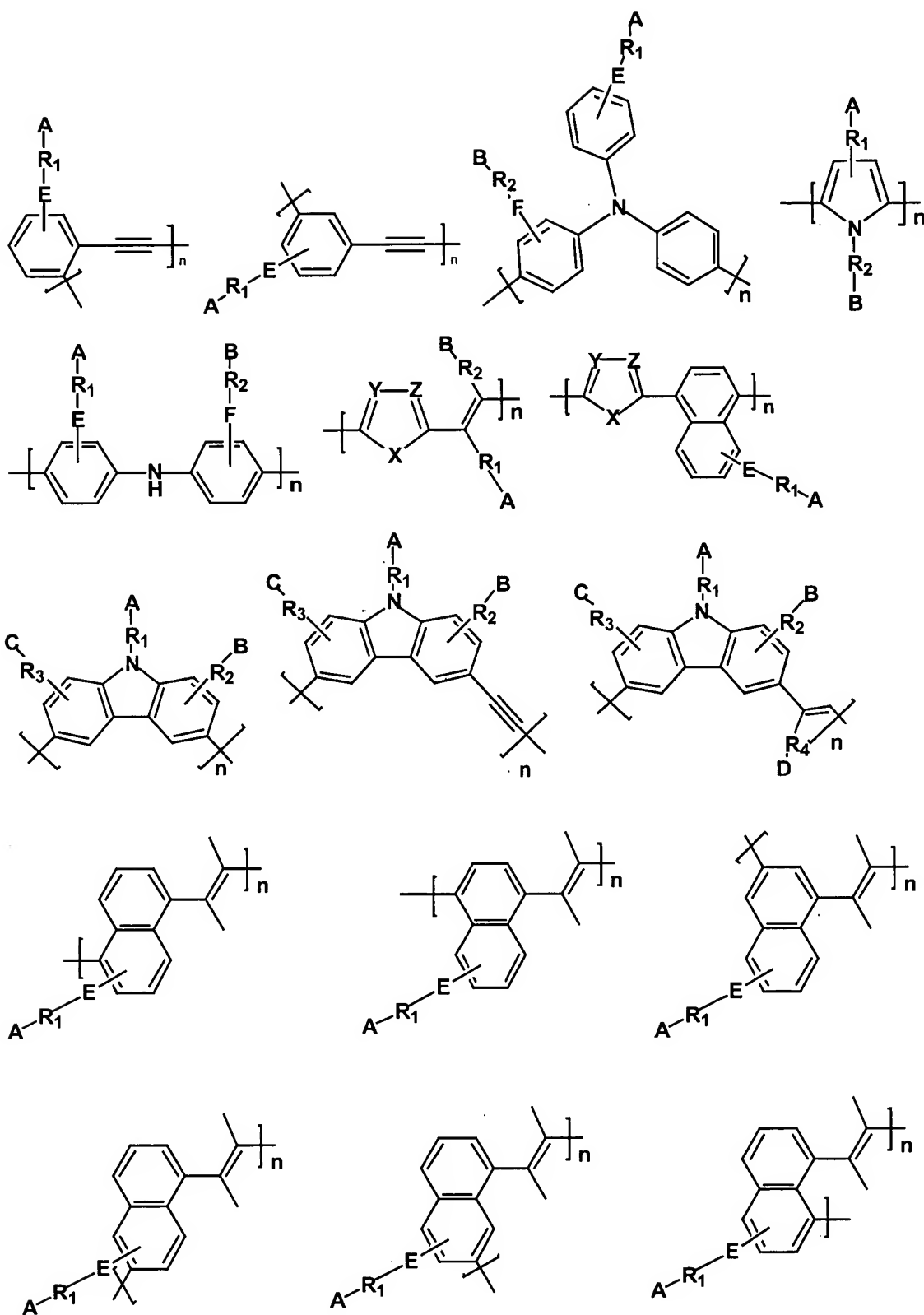


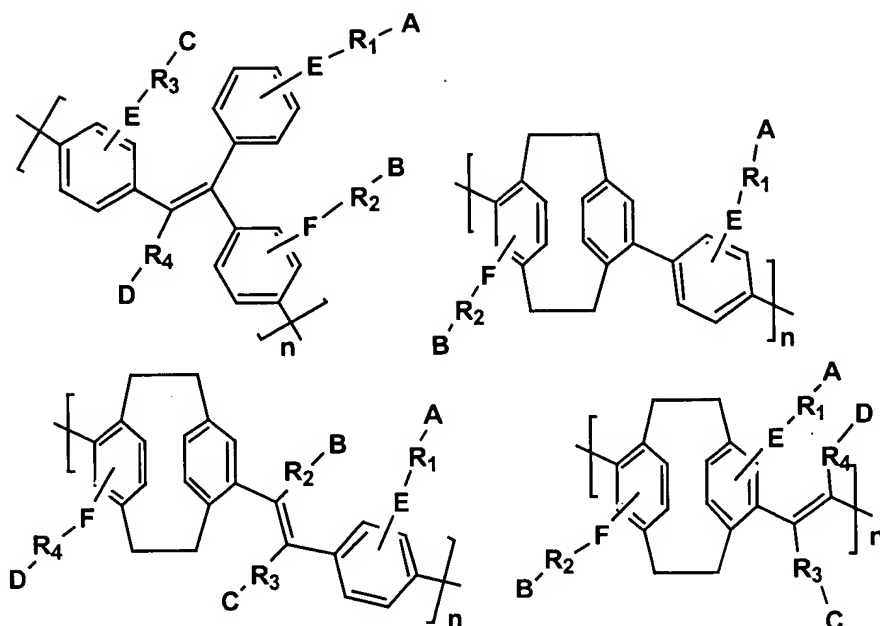
Table 2. Examples of conjugated segments and oligomeric structures of CP



5







In one embodiment, modification of polymer shape was achieved through fractional incorporation of *meta* and *para* linkages on phenylene units adjacent to the fluorenyl monomer units. The *meta/para* ratio is controlled during the polymerization reaction by using of 1,3-phenylenebisboronic acid and 1,4-phenylenebisboronic acid in appropriate ratios. The corresponding polymers may have ratios of *meta* to *para* linkages ranging from 0 to 100%. The introduction of the *meta* linkage not only permits shape control, but also provides the possibility of energy transfer along the polymer main chain, or between different polymer segments, since fragments containing a higher fraction of *para* linkages are of lower energy level, and behave as low energy traps. The synthetic approach is as follows. A neutral polymer is first formed by the Suzuki coupling of a targeted ratio of 1,3-phenylenebisboronic acid and 1,4-phenylenebisboronic acid with 2,7-dibromo-9,9-bis(6'-bromohexyl)fluorene. Conversion to cationic water-soluble polymers is accomplished by addition of condensed trimethylamine. See the examples provided below.

ARTICLES OF MANUFACTURE

The CCPs can be incorporated into any of various articles of manufacture including optoelectronic or electronic devices, biosensors, photodiodes, light-emitting diodes

(“LEDs”), optoelectronic semiconductor chips, semiconductor thin-films, and chips, and can be used in microarray form. The polymer can be incorporated into a polymeric photoswitch. The polymer can be incorporated into an optical interconnect or a transducer to convert a light signal to an electrical impulse. The CCPs can serve as liquid crystal materials. The CCPs, which provide an increased Stoke’s shift resulting from inter- and intramolecular energy transfer, can serve as lasing materials. Flexible CCPs with decreased self-quenching can be used in optoelectronic devices which require more intense emission.

The polymer can be incorporated into articles of manufacture by any suitable technique, including by spin-coating, sequential spin-casting, formation of Langmuir-Blodgett films or electrostatic adsorption techniques.⁸ Articles may be fabricated by stepwise deposition of polyelectrolyte layers; the water solubility of certain flexible CCPs provided herein allows for the sequential deposition of layers of different materials with different solubilities, providing certain advantages during manufacturing, including for the deposition of thin layers of material.

METHODS OF USE OF THE CONFORMATIONALLY FLEXIBLE POLYMERS

The CCPs may be used in methods which screen the CCPs for any property of interest. For example, the CCPs may be tested for binding to a target, for energy transfer to a chromophore, for increased fluorescent efficiency, for decreased self-quenching, for absorbance wavelength, and/or for emission wavelength. Particular targets of interest, which include biomaterials and chemical compounds, can be tested against a plurality of different CCPs comprising such angled linkers to find particular CCPs with properties desirable for use with a given target.

A sensor molecule that is specific for the target may be used in conjugation with the CCP, as can a signaling chromophore to which energy may be transferred from the CCP. The CCP preferably interacts with the target and/or the sensor through electrostatic interactions. Preferably, a sensor of known structure is used to determine the presence and/or amount of the target in the sample. The sensor may provide a signal specific to its

complementary target in any of various ways, including through incorporation of a specific signaling chromophore which can receive energy from the CCP, or through a defined and/or determinable position on a substrate. The signaling chromophore may be incorporated into the sensor, into a substrate, or may be recruited to a complex formed from the sensor and the target. Formation of such a complex results in an increase of energy transfer from a CCP upon excitation to the signaling chromophore, which may be detected directly or indirectly to provide information regarding the target.

Any target molecule and any sensor molecule that can bind to each other can in principle be used, with the proviso that the CCP must bind to or otherwise associate with at least one member of that binding pair or the complex they form; this may be accomplished through electrostatic interaction with negatively charged groups thereon, or by physical proximity by incorporation into a device such as a sensor which also binds to or is associated with one or more members of the complex thereby bringing the CCP into signaling juxtaposition to the signaling chromophore.

The target molecule may be a biomolecule, for example a peptide or protein, a polynucleotide such as DNA or RNA, and an antibody. The target may be a chemical compound, and the CCPs may be incorporated into chemical sensors to detect any species of interest, for example an explosive, e.g. trinitrotoluene.

Similarly, exemplary sensor molecules include chemical compounds and biomolecules. Exemplary sensor biomolecules include a polynucleotide with an anionic backbone such as DNA or RNA, a polynucleotide with an uncharged backbone such as a peptide nucleic acid (PNA), an antibody, and a peptide or protein, which may be a polynucleotide-binding protein (PBP).

When the sensor biomolecule is a polynucleotide, the sensor polynucleotide can be branched, multimeric or circular, but is typically linear, and can contain nonnatural bases. Sensor polynucleotides can be prepared with any desired sequence of bases. Chemical methods for attaching a signaling chromophore to sensor biomolecules are known in the

art.⁹ Specific sensor polynucleotide structures, including structures conjugated to chromophores, can be custom-made using commercial sources or chemically synthesized.

Any protein which can bind to a target polynucleotide of interest can be employed as a PBP. Chemical methods for attaching the signaling chromophore to the sensor PBP

5 are known. Specific sensor PBP structures, including structures conjugated to chromophores, can be custom-made using commercial sources or chemically synthesized.

Non-limiting examples of PBPs include DNA-binding proteins including transcription factors, splicing factors, poly(A) binding proteins, chromatin components, viral proteins, proteins which detect viral infection, replication factors, and proteins involved in mitotic

10 and/or meiotic cell division. RNA-protein interactions mediate important cellular processes including transcription, posttranscriptional modifications, RNA splicing, and translation^{10,11,12,13}. The replication cycle of many pathogenic viruses, such as the human immunodeficiency virus type 1 (HIV-1)¹⁴, picornaviruses¹⁵ and influenza viruses¹⁶, rely on

specific RNA-protein interactions. The specificity of such interactions can be used as the

15 basis for sequence specific sensors for utility in medical diagnostics and genomic studies.

Exemplary polynucleotide binding proteins include zinc-finger proteins, homeodomain proteins, winged-helix (forkhead) proteins, leucine-zipper proteins, helix-loop-helix proteins, helix-turn-helix proteins, and histone-like proteins. The PBPs may be isolated

from a cell source, or may be produced *in vitro*, for example through in vitro

20 transcription/translation methods or through completely synthetic methods. The PBPs can be naturally occurring proteins, mutants of naturally occurring proteins, randomly produced proteins produced, for example, by molecular evolution methods, or suspected polynucleotide binding proteins of unknown binding specificity. Examples of specific

PBP's which can be used include Tat which binds to the Rev Responsive Element of
25 human immunodeficiency virus (HIV), the matrix protein M1 which binds to Type A influenza virus RNA, and hnRNP U protein which binds to pre-ribosomal RNA.

In some cases it may be desirable to add an organic solvent, for example a water miscible organic solvent such as ethanol, to an assay utilizing a CCP to decrease

hydrophobic interactions between the CCP and another component of the assay and thereby reduce background signal.

Where the target is present in a biological sample, the portion of a sample comprising or suspected of comprising the target can be any source of biological material that can be obtained from a living organism directly or indirectly, including cells, tissue or fluid, and the deposits left by that organism, including viruses, mycoplasma, and fossils. The sample may comprise a target polynucleotide prepared through synthetic means, in whole or in part. Typically, the sample is obtained as or dispersed in a predominantly aqueous medium. Nonlimiting examples of the sample include blood, urine, semen, milk, sputum, mucus, a buccal swab, a vaginal swab, a rectal swab, an aspirate, a needle biopsy, a section of tissue obtained for example by surgery or autopsy, plasma, serum, spinal fluid, lymph fluid, the external secretions of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, tumors, organs, samples of *in vitro* cell culture constituents (including but not limited to conditioned medium resulting from the growth of cells in cell culture medium, putatively virally infected cells, recombinant cells, and cell components), and a recombinant library comprising polynucleotide sequences. The sample may be presented on a substrate as described herein. The substrate may be a slide comprising the sample, such as is used in fluorescence *in situ* hybridization (FISH).

The sample can be a positive control sample which is known to contain the target or a surrogate thereof. A negative control sample can also be used which, although not expected to contain the target, is suspected of containing it (via contamination of one or more of the reagents) or another component capable of producing a false positive, and is tested in order to confirm the lack of contamination of the reagents used in a given assay by the target, as well as to determine whether a given set of assay conditions produces false positives (a positive signal even in the absence of target in the sample).

The sample can be diluted, dissolved, suspended, extracted or otherwise treated to solubilize and/or purify any target present or to render it accessible, for example to reagents which are used in an amplification scheme or to detection reagents. Where the sample contains cells, the cells can be lysed or permeabilized to release the polynucleotides within

the cells. One step permeabilization buffers can be used to lyse cells which allow further steps to be performed directly after lysis, for example a polymerase chain reaction.

Detection of target polynucleotides. Where the conformationally flexible polymer is used to detect a target polynucleotide in a sample, the target polynucleotide can be single-stranded, double-stranded, or higher order, and can be linear or circular. Exemplary single-stranded target polynucleotides include mRNA, rRNA, tRNA, hnRNA, ssRNA or ssDNA viral genomes, although these polynucleotides may contain internally complementary sequences and significant secondary structure. Exemplary double-stranded target polynucleotides include genomic DNA, mitochondrial DNA, chloroplast DNA, dsRNA or dsDNA viral genomes, plasmids, phage, and viroids. The target polynucleotide can be prepared synthetically or purified from a biological source. The target polynucleotide may be purified to remove or diminish one or more undesired components of the sample or to concentrate the target polynucleotide. Conversely, where the target polynucleotide is too concentrated for the particular assay, the target polynucleotide may be diluted.

Following sample collection and optional nucleic acid extraction, the nucleic acid portion of the sample comprising the target polynucleotide can be subjected to one or more preparative reactions. These preparative reactions can include *in vitro* transcription (IVT), labeling, fragmentation, amplification and other reactions. mRNA can first be treated with reverse transcriptase and a primer to create cDNA prior to detection and/or amplification; this can be done *in vitro* with purified mRNA or *in situ*, e.g. in cells or tissues affixed to a slide. A variety of amplification methods are suitable for use; nonlimiting examples of suitable amplification reactions include the polymerase chain reaction method (PCR), the ligase chain reaction (LCR), self sustained sequence replication (3SR), nucleic acid sequence-based amplification (NASBA), the use of Q Beta replicase, reverse transcription, nick translation, and the like.

The target polynucleotide can be typically amplified by contacting one or more strands of the target polynucleotide with a primer and a polymerase having suitable activity to extend the primer and copy the target polynucleotide to produce a full-length

complementary polynucleotide or a smaller portion thereof. Any enzyme having a polymerase activity which can copy the target polynucleotide can be used, including DNA polymerases, RNA polymerases, reverse transcriptases, enzymes having more than one type of polymerase activity, and the enzyme can be thermolabile or thermostable. Mixtures
5 of enzymes can also be used.

Suitable reaction conditions are chosen to permit amplification of the target polynucleotide, including pH, buffer, ionic strength, presence and concentration of one or more salts, presence and concentration of reactants and cofactors such as nucleotides and magnesium and/or other metal ions (e.g., manganese), optional cosolvents, temperature,
10 thermal cycling profile for amplification schemes comprising a polymerase chain reaction, and may depend in part on the polymerase being used as well as the nature of the sample. Cosolvents include formamide (typically at from about 2 to about 10 %), glycerol (typically at from about 5 to about 10 %), and DMSO (typically at from about 0.9 to about 10 %). Techniques may be used in the amplification scheme in order to minimize the
15 production of false positives or artifacts produced during amplification. These include "touchdown" PCR, hot-start techniques, use of nested primers, or designing PCR primers so that they form stem-loop structures in the event of primer-dimer formation and thus are not amplified. Techniques to accelerate PCR can be used, for example centrifugal PCR, which allows for greater convection within the sample, and comprising infrared heating
20 steps for rapid heating and cooling of the sample. One or more cycles of amplification can be performed.

Amplified target polynucleotides may be subjected to post amplification treatments. For example, in some cases, it may be desirable to fragment the target polynucleotide prior to hybridization in order to provide segments which are more readily accessible.
25 Fragmentation of the nucleic acids can be carried out by any method producing fragments of a size useful in the assay being performed; suitable physical, chemical and enzymatic methods are known in the art.

An amplification reaction can be performed under conditions which allow a sensor polynucleotide to hybridize to the amplification product during at least part of an

amplification cycle. When the assay is performed in this manner, real-time detection of this hybridization event can take place by monitoring for a change in light emission from the signaling chromophore that occurs upon such hybridization during the amplification scheme.

5 *Signaling Chromophores.* Chromophores useful in the methods described herein include any substance which can absorb energy from a flexible CCP and emit light. Chemical methods for attaching a signaling chromophore to a sensor molecule or other assay component are known.¹⁷ For multiplexed assays, a plurality of different signaling chromophores can be used with detectably different emission spectra. The chromophore
10 can be a lumophore or a fluorophore. Typical fluorophores include fluorescent dyes, semiconductor nanocrystals, lanthanide chelates, polynucleotide-specific dyes and green fluorescent protein.

Exemplary fluorescent dyes include fluorescein, 6-FAM, rhodamine, Texas Red, tetramethylrhodamine, carboxyrhodamine, carboxyrhodamine 6G, carboxyrhodol,
15 carboxyrhodamine 110, Cascade Blue, Cascade Yellow, coumarin, Cy2®, Cy3®, Cy3.5®, Cy5®, Cy5.5®, Cy-Chrome, phycoerythrin, PerCP (peridinin chlorophyll-a Protein), PerCP-Cy5.5, JOE (6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein), NED, ROX (5-(and-6)-carboxy-X-rhodamine), HEX, Lucifer Yellow, Marina Blue, Oregon Green 488, Oregon Green 500, Oregon Green 514, Alexa Fluor® 350, Alexa Fluor® 430, Alexa
20 Fluor® 488, Alexa Fluor® 532, Alexa Fluor® 546, Alexa Fluor® 568, Alexa Fluor® 594, Alexa Fluor® 633, Alexa Fluor® 647, Alexa Fluor® 660, Alexa Fluor® 680, 7-amino-4-methylcoumarin-3-acetic acid, BODIPY® FL, BODIPY® FL-Br₂, BODIPY® 530/550, BODIPY® 558/568, BODIPY® 564/570, BODIPY® 576/589, BODIPY® 581/591, BODIPY® 630/650, BODIPY® 650/665, BODIPY® R6G, BODIPY® TMR, BODIPY®
25 TR, conjugates thereof, and combinations thereof. Exemplary lanthanide chelates include europium chelates, terbium chelates and samarium chelates.

A wide variety of fluorescent semiconductor nanocrystals ("SCNCs") are known in the art; methods of producing and utilizing semiconductor nanocrystals are described in: PCT Publ. No. WO 99/26299 published May 27, 1999, inventors Bawendi et al.; USPN

5,990,479 issued Nov. 23, 1999 to Weiss et al.; and Bruchez et al., Science 281:2013, 1998. Semiconductor nanocrystals can be obtained with very narrow emission bands with well-defined peak emission wavelengths, allowing for a large number of different SCNCs to be used as signaling chromophores in the same assay, optionally in combination with
5 other non-SCNC types of signaling chromophores.

Exemplary polynucleotide-specific dyes include acridine orange, acridine homodimer, actinomycin D, 7-aminoactinomycin D (7-AAD), 9-amino-6-chloro-2-methoxyacridine (ACMA), BOBO™-1 iodide (462/481), BOBO™-3 iodide (570/602), BO-PRO™-1 iodide (462/481), BO-PRO™-3 iodide (575/599), 4',6-diamidino-2-
10 phenylindole, dihydrochloride (DAPI), 4',6-diamidino-2-phenylindole, dihydrochloride (DAPI), 4',6-diamidino-2-phenylindole, dilactate (DAPI, dilactate), dihydroethidium (hydroethidine), dihydroethidium (hydroethidine), dihydroethidium (hydroethidine), ethidium bromide, ethidium diazide chloride, ethidium homodimer-1 (EthD-1), ethidium homodimer-2 (EthD-2), ethidium monoazide bromide (EMA), hexidium iodide, Hoechst
15 33258, Hoechst 33342, Hoechst 34580, Hoechst S769121, hydroxystilbamidine, methanesulfonate, JOJO™-1 iodide (529/545), JO-PRO™-1 iodide (530/546), LOLO™-1 iodide (565/579), LO-PRO™-1 iodide (567/580), NeuroTrace™ 435/455, NeuroTrace™ 500/525, NeuroTrace™ 515/535, NeuroTrace™ 530/615, NeuroTrace™ 640/660, OliGreen, PicoGreen® ssDNA, PicoGreen® dsDNA, POPO™-1 iodide (434/456),
20 POPO™-3 iodide (534/570), PO-PRO™-1 iodide (435/455), PO-PRO™-3 iodide (539/567), propidium iodide, RiboGreen®, *SlowFade*®, *SlowFade*® Light, SYBR® Green I, SYBR® Green II, SYBR® Gold, SYBR® 101, SYBR® 102, SYBR® 103, SYBR® DX, TO-PRO®-1, TO-PRO®-3, TO-PRO®-5, TOTO®-1, TOTO®-3, YO-PRO®-1 (oxazole yellow), YO-PRO®-3, YOYO®-1, YOYO®-3, TO, SYTOX® Blue, SYTOX® Green,
25 SYTOX® Orange, SYTO® 9, SYTO® BC, SYTO® 40, SYTO® 41, SYTO® 42, SYTO® 43, SYTO® 44, SYTO® 45, SYTO® Blue, SYTO® 11, SYTO® 12, SYTO® 13, SYTO® 14, SYTO® 15, SYTO® 16, SYTO® 20, SYTO® 21, SYTO® 22, SYTO® 23, SYTO® 24, SYTO® 25, SYTO® Green, SYTO® 80, SYTO® 81, SYTO® 82, SYTO® 83, SYTO® 84, SYTO® 85, SYTO® Orange, SYTO® 17, SYTO® 59, SYTO® 60, SYTO®
30 61, SYTO® 62, SYTO® 63, SYTO® 64, SYTO® Red, netropsin, distamycin, acridine

orange, 3,4-benzopyrene, thiazole orange, TOMEHE, daunomycin, acridine, pentyl-TOTAB, and butyl-TOTIN. Asymmetric cyanine dyes may be used as the polynucleotide-specific dye. Other dyes of interest include those described by Geierstanger, B.H. and Wemmer, D.E., *Annu. Rev. Vioshys. Biomol. Struct.* 1995, 24, 463-493, by Larson, C.J. and Verdine, G.L., *Bioorganic Chemistry: Nucleic Acids*, Hecht, S.M., Ed., Oxford University Press: New York, 1996; pp 324-346, and by Glumoff, T. and Goldman, A. *Nucleic Acids in Chemistry and Biology*, 2nd ed., Blackburn, G.M. and Gait, M.J., Eds., Oxford University Press: Oxford, 1996, pp375-441. The polynucleotide-specific dye may be an intercalating dye, and may be specific for double-stranded polynucleotides. Other dyes and fluorophores are described at www.probes.com (Molecular Probes, Inc.).

The term "green fluorescent protein" refers to both native *Aequorea* green fluorescent protein and mutated versions that have been identified as exhibiting altered fluorescence characteristics, including altered excitation and emission maxima, as well as excitation and emission spectra of different shapes (Delagrave, S. et al. (1995) *Bio/Technology* 13:151-154; Heim, R. et al. (1994) *Proc. Natl. Acad. Sci. USA* 91:12501-12504; Heim, R. et al. (1995) *Nature* 373:663-664). Delagrave et al. isolated mutants of cloned *Aequorea victoria* GFP that had red-shifted excitation spectra. Heim, R. et al. reported a mutant (Tyr66 to His) having a blue fluorescence.

In one variation, a second signaling chromophore, which may be directly or indirectly attached to another of the assay components and/or to a substrate, is used to receive energy from the initial signaling chromophore. In particular applications, this can provide for significant additional selectivity. For example, a polynucleotide-specific dye can be used as either the initial or second signaling chromophore, and may be specific for double-stranded sequences. Energy can then be transferred from the excited conformationally flexible CCP to the initial signaling chromophore, which subsequently transfers energy to the second signaling chromophore, in an overall format that is selective for the target. This cascade of signaling chromophores can, in principle, be extended to use any number of signaling chromophores with compatible absorption and emission profiles. In one embodiment of this variation, an intercalating dye that is specific for double-

stranded polynucleotides is used as the second signaling chromophore, and an initial signaling chromophore that is capable of transferring energy to the second signaling chromophore is conjugated to a sensor polynucleotide. The intercalating dye provides the added selective requirement that the sensor and target polynucleotides hybridize before it is recruited to the detection complex. In the presence of target, the duplex is formed, the dye is recruited, and excitation of the multichromophore leads to signaling from the second signaling chromophore.

Substrates. The methods described herein can be performed on a substrate in any of a variety of formats. One or more of the assay components may be incorporated in, attached to, or otherwise associated with the substrate, directly or indirectly. The substrate can comprise a wide range of material, either biological, nonbiological, organic, inorganic, or a combination of any of these. For example, the substrate may be a polymerized Langmuir Blodgett film, functionalized glass, Si, Ge, GaAs, GaP, SiO₂, SiN₄, modified silicon, or any one of a wide variety of gels or polymers such as (poly)tetrafluoroethylene, (poly)vinylidenedifluoride, polystyrene, cross-linked polystyrene, polyacrylic, polylactic acid, polyglycolic acid, poly(lactide coglycolide), polyanhydrides, poly(methyl methacrylate), poly(ethylene-co-vinyl acetate), polysiloxanes, polymeric silica, latexes, dextran polymers, epoxies, polycarbonates, agarose, poly(acrylamide) or combinations thereof. Conducting polymers and photoconductive materials can be used.

Substrates can be planar crystalline substrates such as silica based substrates (e.g. glass, quartz, or the like), or crystalline substrates used in, e.g., the semiconductor and microprocessor industries, such as silicon, gallium arsenide, indium doped GaN and the like, and includes semiconductor nanocrystals.

The substrate can take the form of a photodiode, an optoelectronic sensor such as an optoelectronic semiconductor chip or optoelectronic thin-film semiconductor, or a biochip. The location(s) of the individual sensor(s) on the substrate can be addressable; this can be done in highly dense formats, and the location(s) can be microaddressable or nanoaddressable.

Silica aerogels can also be used as substrates, and can be prepared by methods known in the art. Aerogel substrates may be used as free standing substrates or as a surface coating for another substrate material.

5 The substrate can take any form and typically is a plate, slide, bead, pellet, disk, particle, microparticle, nanoparticle, strand, precipitate, optionally porous gel, sheets, tube, sphere, container, capillary, pad, slice, film, chip, multiwell plate or dish, optical fiber, etc. The substrate can be any form that is rigid or semi-rigid. The substrate may contain raised or depressed regions on which a sensor molecule and/or other assay component is located. The surface of the substrate can be etched using well known techniques to provide for
10 desired surface features, for example trenches, v-grooves, mesa structures, or the like.

Surfaces on the substrate can be composed of the same material as the substrate or can be made from a different material, and can be coupled to the substrate by chemical or physical means. Such coupled surfaces may be composed of any of a wide variety of materials, for example, polymers, plastics, resins, polysaccharides, silica or silica-based
15 materials, carbon, metals, inorganic glasses, membranes, or any of the above-listed substrate materials. The surface can be optically transparent and can have surface Si-OH functionalities, such as those found on silica surfaces.

The substrate and/or its optional surface are chosen to provide appropriate optical characteristics for the synthetic and/or detection methods used. The substrate and/or
20 surface can be transparent to allow the exposure of the substrate by light applied from multiple directions. The substrate and/or surface may be provided with reflective "mirror" structures to increase the recovery of light.

The substrate and/or its surface is generally resistant to, or is treated to resist, the conditions to which it is to be exposed in use, and can be optionally treated to remove any
25 resistant material after exposure to such conditions.

Sensor molecules can be fabricated on or attached to the substrate by any suitable method, for example the methods described in U.S. Pat. No. 5,143,854, PCT Publ. No. WO 92/10092, U.S. Patent Application Ser. No. 07/624,120, filed Dec. 6, 1990 (now

abandoned), Fodor et al., Science, 251: 767-777 (1991), and PCT Publ. No. WO 90/15070). Techniques for the synthesis of these arrays using mechanical synthesis strategies are described in, e.g., PCT Publication No. WO 93/09668 and U.S. Pat. No. 5,384,261.

5 Still further techniques include bead based techniques such as those described in PCT Appl. No. PCT/US93/04145 and pin based methods such as those described in U.S. Pat. No. 5,288,514.

Additional flow channel or spotting methods applicable to attachment of sensor molecules to the substrate are described in U. S. Patent Application Ser. No. 07/980,523, filed Nov. 20, 1992, and U.S. Pat. No. 5,384,261. Reagents are delivered to the substrate
10 by either (1) flowing within a channel defined on predefined regions or (2) "spotting" on predefined regions. A protective coating such as a hydrophilic or hydrophobic coating (depending upon the nature of the solvent) can be used over portions of the substrate to be protected, sometimes in combination with materials that facilitate wetting by the reactant solution in other regions. In this manner, the flowing solutions are further prevented from
15 passing outside of their designated flow paths.

Typical dispensers include a micropipette optionally robotically controlled, an ink-jet printer, a series of tubes, a manifold, an array of pipettes, or the like so that various reagents can be delivered to the reaction regions sequentially or simultaneously.

20 EXCITATION AND DETECTION OF THE CHROMOPHORES

Any instrument that provides a wavelength that can excite the conformationally flexible CCP and is shorter than the emission wavelength(s) to be detected can be used for excitation. The excitation source preferably does not significantly excite the signaling chromophore directly. The source may be: a broadband UV light source such as a
25 deuterium lamp with an appropriate filter, the output of a white light source such as a xenon lamp or a deuterium lamp after passing through a monochromator to extract out the desired wavelengths, a continuous wave (cw) gas laser, a solid state diode laser, or any of the pulsed lasers. The emitted light from the signaling chromophore can be detected

through any suitable device or technique; many suitable approaches are known in the art. For example, a fluorometer or spectrophotometer may be used to detect whether the test sample emits light of a wavelength characteristic of the signaling chromophore upon excitation of the CCP.

5

KITS

Kits comprising reagents useful for performing the methods of the invention are also provided. In one embodiment, a kit comprises a sensor molecule that can bind to a target molecule of interest and a conformationally flexible CCP. The sensor molecule may be conjugated to a signaling chromophore. In the presence of the target in the sample, the sensor binds to the target, resulting in increased emission of energy from the signaling chromophore, which can be detected.

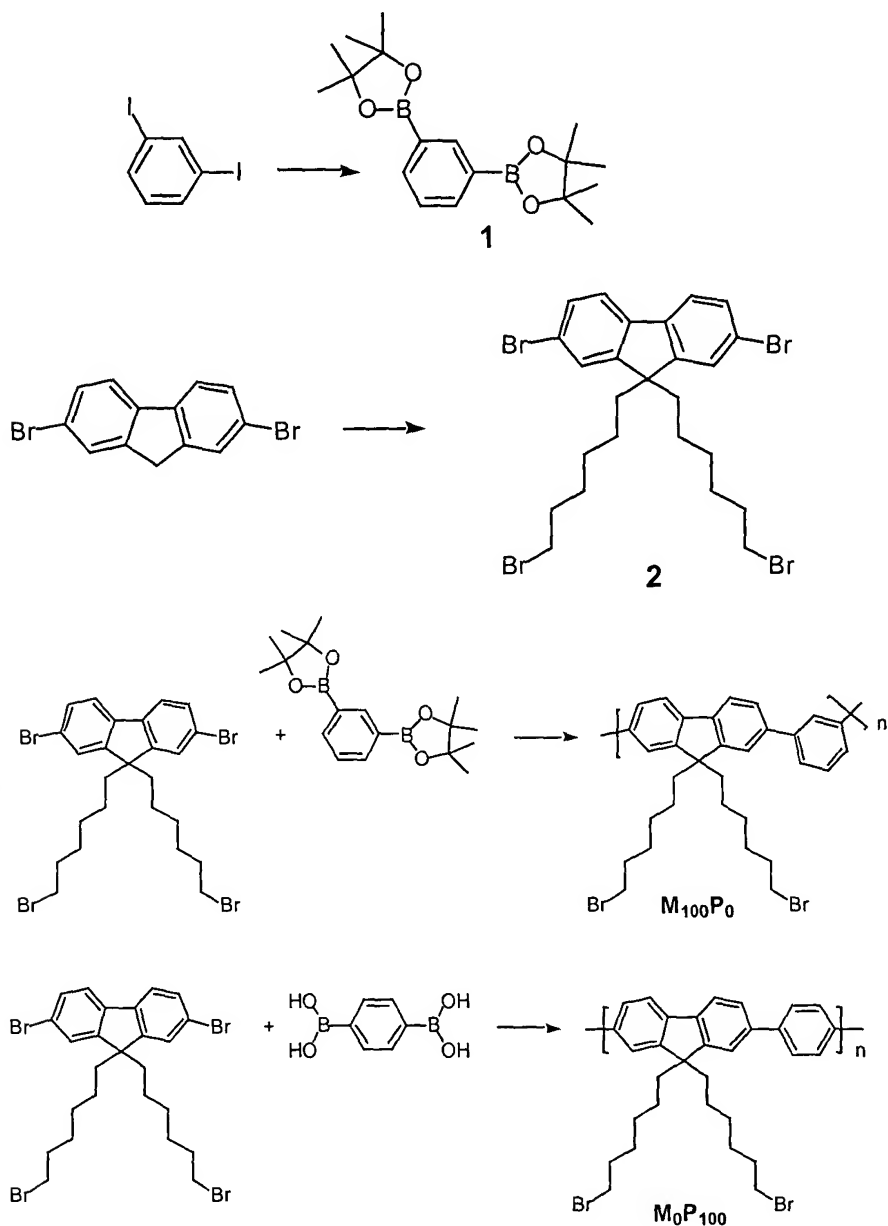
The components of the kit are retained by a housing. Instructions for using the kit to perform a method of the invention can be provided with the housing, and can be provided in any fixed medium. The instructions may be located inside the housing or outside the housing, and may be printed on the interior or exterior of any surface forming the housing which renders the instructions legible. The kit may be in multiplex form, containing pluralities of one or more different sensor molecules which can bind to corresponding different target molecules.

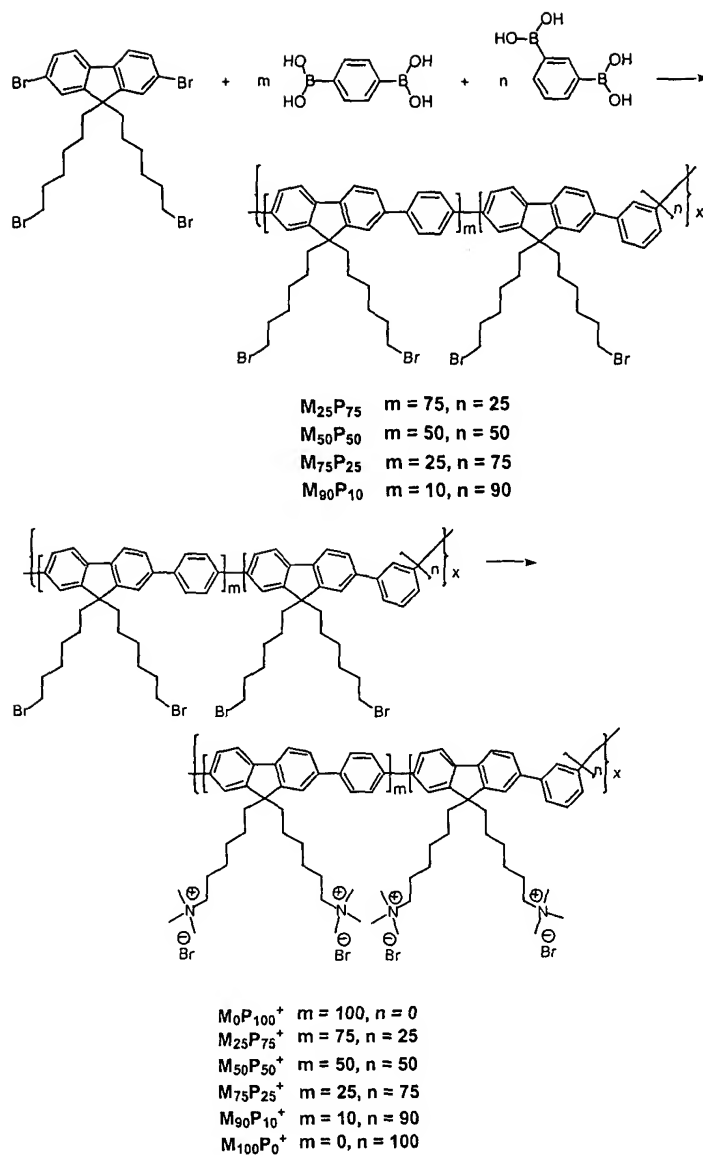
20

EXAMPLES

The following examples are set forth so as to provide those of ordinary skill in the art with a complete description of how to make and use the present invention, and are not intended to limit the scope of what is regarded as the invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) but some experimental error and deviation should be accounted for. Unless otherwise indicated, parts are parts by weight, temperature is degree centigrade and pressure is at or near atmospheric, and all materials are commercially available.

In one embodiment, conformationally flexible cationic water-soluble conjugated polymers were synthesized through the Suzuki coupling reaction and a post-polymerization quaternization step. Synthetic examples are given with respect to the specific polymers under Formula 1. The synthetic routes are shown in Scheme 1.





Scheme 1. Synthetic procedure for cationic water-soluble polymers.

An overview of the method is as follows. 1,3-Bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan)phenylene (1) was obtained in 46% yield by treating 1,3-diiodobenzene with bis(pinacolato)diborane in the presence of $PdCl_2(dppf)$ and potassium acetate in DMSO. 2,7-Dibromo-9,9-bis(6'-bromohexyl)fluorene (2) was obtained by the treatment of 2,7-dibromofluorene with 50% KOH, followed by addition of excess of 1,6-dibromohexane in 85% yield. Coupling of one equivalent of the dibromide monomer with one net equivalent of diboronic acid or diboronic ester, under Suzuki coupling conditions using $PdCl_2(dppf)$ in

refluxing THF/H₂O for 24 h, followed by purification gave the desired polymers in 39% to 88% yield. The products were thoroughly washed with methanol and acetone, and then dried in vacuum for 24 h. Formation of the water-soluble polymers was achieved by stirring the polymer in condensed trimethylamine in a THF/H₂O solvent mixture for 24 h.

5 **Example 1.** 1,3-Bis(4,4,5,5,-tetramethyl-1,3,2-dioxaborolan)phenylene (**1**).

A flask charged with 1,3-diiodobenzene (1.0 g, 3 mmol), bis(pinacolato)diborane (2.3 g, 9 mmol), potassium acetate (2.1 g, 21 mmol), PdCl₂(dppf) (150 mg, 0.18 mmol), and 15 mL of anhydrous DMSO was degassed for 15 minutes. The mixture was stirred at 80 °C for 12 h, cooled to room temperature and then poured into 100 mL of ice water. The mixture was extracted with CHCl₃, and the combined organic layers were dried over anhydrous MgSO₄. After the solvent was evaporated, the residue was purified by chromatography using silica gel (Hexane:CHCl₃ = 1:1) and then recrystallized from ethanol to afford **1** (460 mg, 46%) as a white solid. ¹H NMR (200 MHz, CDCl₃): δ 8.28 (s, 1H), 7.91-7.89 (d, 2H), 7.38 (t, 1H), 1.35 (s, 24H). ¹³C NMR (50 MHz, CDCl₃): δ 141.4, 137.8, 127.3, 83.9, 25.1.

15 **Example 2.** 2,7-Dibromo-9,9-bis(6'-bromohexyl)fluorene (**2**).

To a mixture of tetrabutylammonium bromide (300 mg, 9.3 mmol), aqueous potassium hydroxide (100 mL, 50%) and 1,6-dibromohexane (22.6 g, 92.6 mmol) was added 2,7-dibromofluorene at 75 °C. After 15 minutes, the mixture was cooled down to room temperature, and extracted with CH₂Cl₂. The organic layer was washed with water, aqueous HCl, water and brine, dried over MgSO₄, and then concentrated. Unreacted 1,6-dibromohexane was distilled off. The residue was purified by silica gel column chromatography (Hexane:CHCl₃ = 9:1) and recrystallized from ethanol to give **2** (4.8 g, 80%) as a white solid. ¹H NMR (200 MHz, CDCl₃): δ 7.2-7.4 (m, 6H), 3.12 (t, 4H), 1.75 (t, 4H), 1.5 (m, 4H), 1.0 (m, 8H), 0.4 (m, 4H). ¹³C NMR (50 MHz, CDCl₃): δ 152.3, 139.2, 130.5, 126.2, 121.7, 121.4, 55.7, 40.2, 34.1, 32.8, 29.1, 27.9, 23.6.

25 **Example 3.** Poly(9,9-bis(6'-bromohexyl)fluorene-co-alt-1,3 -phenylene) (M₁₀₀P₀).

2,7-Dibromo-9,9-bis(6'-bromohexyl)fluorene (325 mg, 0.5 mmol), 1,3-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan)phenylene (166 mg, 0.5 mmol), Pd(PPh₃)₄ (8 mg) and

potassium carbonate (830 mg, 6 mmol) were placed in a 25 mL round bottle flask. A mixture of water (3 mL) and toluene (5 mL) was added to the flask. After degassing, the mixture was refluxed at 85 °C for 20 h, and then precipitated into methanol. The polymer was filtered and washed with methanol and acetone, and then dried in vacuum for 24 h to afford $M_{100}P_0$ (251 mg, 88%) as a light yellow solid. 1H NMR (200 MHz, $CDCl_3$): δ 7.9-7.6 (m, 10H), 3.3-3.2 (t, 4H), 2.1 (m, 4H), 1.7-1.6 (m, 4H), 1.3-1.2 (m, 8H), 0.8 (m, 4H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 152.1, 142.9, 140.9, 130.1, 129.5, 128.0, 126.8, 122.5, 120.9, 55.9, 40.9, 34.5, 33.3, 29.7, 28.5, 24.3. GPC (THF, polystyrene standard), M_w : 40,250 g/mol; M_n : 14,980 g/mol; PDI: 2.8. UV-vis ($CHCl_3$): λ_{max} = 337 nm; PL ($CHCl_3$): λ_{max} = 363 nm.

Example 4. Poly(9,9-bis(6'-bromohexyl)fluorene-co-alt-1,4-phenylene) (M_0P_{100}). 2,7-Dibromo-9,9-bis(6'-bromohexyl)fluorene (325 mg, 0.5 mmol), 1,4-phenylenebisboronic acid (82.9 mg, 0.5 mmol), Pd(dppf) Cl_2 (7 mg) and potassium carbonate (830mg, 6 mmol) were placed in a 25 mL round bottle flask. A mixture of water (3 mL) and THF (6 mL) was added to the flask and degassed. The mixture was refluxed at 85 °C for 24 h, and then precipitated into methanol. The polymer was filtered and washed with methanol and acetone, and then dried in vacuum for 24 h to afford M_0P_{100} (220 mg, 78%) as an off-white solid. 1H NMR (200 MHz, $CDCl_3$): δ 7.8 (m, 5H), 7.7-7.6 (m, 4H), 7.5 (m, 1H), 3.3 (t, 4H), 2.1 (m, 4H), 1.7 (m, 4H), 1.3-1.2 (m, 8H), 0.8 (m, 4H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 151.9, 140.9, 140.7, 140.2, 128.1, 126.6, 121.8, 120.8, 55.7, 40.9, 34.5, 33.2, 29.6, 28.3, 24.2. GPC (THF, polystyrene standard), M_w : 25,850 g/mol; M_n : 12,840 g/mol; PDI: 2.0. UV-vis ($CHCl_3$): λ_{max} = 372 nm; PL ($CHCl_3$): λ_{max} = 408 nm.

Example 5. Random copolymer $M_{25}P_{75}$. 2,7-Dibromo-9,9-bis(6'-bromohexyl)fluorene (325 mg, 0.5 mmol), 1,4-phenylenebisboronic acid (62.2 mg, 0.375 mmol), 1,3-phenylenebisboronic acid (20.7 mg, 0.125 mmol), Pd(dppf) Cl_2 (7 mg) and potassium carbonate (830mg, 6 mmol) were placed in a 25 mL round bottle flask. A mixture of water (3 mL) and THF (6 mL) was added to the flask and degassed. The mixture was refluxed at 85 °C for 24 h, and then precipitated into methanol. The polymer was filtered and washed with methanol and acetone, and then dried

in vacuum for 24 h to afford $M_{25}P_{75}$ (248 mg, 88%) as an off-white solid. ^1H NMR (200 MHz, CDCl_3): δ 7.9-7.6 (m, 10H), 3.3-3.2 (t, 4H), 2.1 (m, 4H), 1.7-1.6 (m, 4H), 1.3-1.2 (m, 8H), 0.8 (m, 4H). GPC (THF, polystyrene standard), M_w : 29,000 g/mol; M_n : 14,720 g/mol; PDI: 1.9. UV-vis (CHCl_3): λ_{max} = 365 nm; PL (CHCl_3): λ_{max} = 407 nm.

5 **Example 6.** Random copolymer $M_{50}P_{50}$.

2,7-Dibromo-9,9-bis(6'-bromohexyl)fluorene (325 mg, 0.5 mmol), 1,4-phenylenebisboronic acid (41.5 mg, 0.25 mmol), 1,3-phenylenebisboronic acid (41.5 mg, 0.25 mmol), $\text{Pd}(\text{dppf})\text{Cl}_2$ (7 mg) and potassium carbonate (830mg, 6 mmol) were placed in a 25 mL round bottle flask. A mixture of water (3 mL) and THF (6 mL) was added to the
10 flask and degassed. The mixture was refluxed at 85 °C for 24 h, and then precipitated into methanol. The polymer was filtered and washed with methanol and acetone, and then dried in vacuum for 24 h to afford $M_{50}P_{50}$ (220 mg, 78%) as an off-white solid. ^1H NMR (200 MHz, CDCl_3): δ 7.9-7.6 (m, 10H), 3.3-3.2 (t, 4H), 2.1 (m, 4H), 1.7-1.6 (m, 4H), 1.3-1.2 (m, 8H), 0.8 (m, 4H). GPC (THF, polystyrene standard), M_w : 17,340 g/mol; M_n : 10,080 g/mol; PDI: 1.7. UV-vis (CHCl_3): λ_{max} = 351 nm; PL (CHCl_3): λ_{max} = 405 nm.
15

Example 7. Random copolymer $M_{75}P_{25}$.

2,7-Dibromo-9,9-bis(6'-bromohexyl)fluorene (325 mg, 0.5 mmol), 1,4-phenylenebisboronic acid (20.7 mg, 0.125 mmol), 1,3-phenylenebisboronic acid (62.2 mg, 0.375 mmol), $\text{Pd}(\text{dppf})\text{Cl}_2$ (7 mg) and potassium carbonate (830mg, 6 mmol) were placed
20 in a 25 mL round bottle flask. A mixture of water (3 mL) and THF (6 mL) was added to the flask. After degassing, the mixture was refluxed at 85 °C for 24 h, and then precipitated into methanol. The polymer was filtered and washed with methanol and acetone, and then dried in vacuum for 24 h to afford $M_{75}P_{25}$ (130 mg, 46%) as an off-white solid. ^1H NMR (200 MHz, CDCl_3): δ 7.9-7.6 (m, 10H), 3.3-3.2 (t, 4H), 2.1 (m, 4H), 1.7-1.6 (m, 4H), 1.3-
25 1.2 (m, 8H), 0.8 (m, 4H). GPC (THF, polystyrene standard), M_w : 13,000 g/mol; M_n : 8,700 g/mol; PDI: 1.4. UV-vis (CHCl_3): λ_{max} = 342 nm; PL (CHCl_3): λ_{max} = 400 nm.

Example 8. Random copolymer $M_{90}P_{10}$.

2,7-Dibromo-9,9-bis(6'-bromohexyl)fluorene (325 mg, 0.5 mmol), 1,4-phenylenebisboronic acid (8 mg, 0.05 mmol), 1,3-phenylenebisboronic acid (75 mg, 0.45

mmol), Pd(dppf)Cl₂ (7 mg) and potassium carbonate (830mg, 6 mmol) were placed in a 25 mL round bottle flask. A mixture of water (3 mL) and THF (6 mL) was added to the flask and degassed. The mixture was refluxed at 85 °C for 24 h, and then precipitated into methanol. The polymer was filtered and washed with methanol and acetone and then dried in vacuum for 24 h to afford M₉₀P₁₀ (110 mg, 39%) as an off-white solid. ¹H NMR (200 MHz, CDCl₃): δ 7.9-7.6 (m, 10H), 3.3-3.2 (t, 4H), 2.1 (m, 4H), 1.7-1.6 (m, 4H), 1.3-1.2 (m, 8H), 0.8 (m, 4H). GPC (THF, polystyrene standard), M_w: 8,400 g/mol; M_n: 5,800 g/mol; PDI: 1.4. UV-vis (CHCl₃): λ_{max} = 338 nm; PL (CHCl₃): λ_{max} = 400 nm.

Example 9. Poly(9,9-bis(6'-N,N,N,-trimethylammonium)hexyl)fluorene-co-alt-1,3-phenylene) dibromide (M₁₀₀P₀⁺).

Condensed trimethylamine (2 mL) was added dropwise to a solution of the neutral polymer M₁₀₀P₀ (60 mg) in THF (10 mL) at -78 °C. The mixture was allowed to warm up to room temperature. The precipitate was re-dissolved by the addition of water (10 mL). After the mixture was cooled down to -78 °C, extra trimethylamine (2 mL) was added and the mixture was stirred for 24 h at room temperature. After removing most of the solvent, acetone was added to precipitate M₁₀₀P₀⁺ (63 mg, 78%) as a light yellow powder. ¹H NMR (500 MHz, CD₃OD): δ 8.1-7.7 (m, 10H), 3.3-3.2 (t, 4H), 3.1 (s, 18H), 2.3 (br, 4H), 1.6 (br, 4H), 1.3 (br, 8H), 0.8 (br, 4H). ¹³C NMR (125 MHz, CD₃OD): δ 151.9, 142.4, 140.9, 140.6, 129.77, 126.5, 126.1, 125.6, 121.6, 120.5, 66.7, 55.7, 52.5, 40.1, 29.2, 25.8, 23.8, 22.6. UV-vis (H₂O): λ_{max} = 334 nm; PL (H₂O): λ_{max} = 369 nm. ε = 3.69×10⁴ M⁻¹cm⁻¹ per monomer unit.

Example 10. Poly(9,9-bis(6'-N,N,N,-trimethylammonium)hexyl)fluorene-co-alt-1,4-phenylene) dibromide (M₀P₁₀₀⁺).

Condensed trimethylamine (2 mL) was added dropwise to a solution of the neutral polymer M₀P₁₀₀ (60 mg) in THF (10 mL) at -78 °C. The mixture was allowed to warm up to room temperature. The precipitate was re-dissolved by the addition of water (10 mL). After the mixture was cooled down to -78 °C, extra trimethylamine (2 mL) was added and the mixture was stirred for 24 h at room temperature. After removing most of the solvent, acetone was added to precipitate M₀P₁₀₀⁺ (72 mg, 89%) as an off-white powder. ¹H NMR

(500 MHz, CD₃OD): δ 8.0-7.8 (m, 10H), 3.3-3.2 (t, 4H), 3.1 (s, 18H), 2.3 (br, 4H), 1.6 (br, 4H), 1.3 (br, 8H), 0.8 (br, 4H). ¹³C NMR (125 MHz, CD₃OD): δ 151.8, 140.9, 140.4, 140.0, 127.6, 126.1, 121.2, 120.5, 66.7, 55.7, 52.5, 40.2, 29.2, 25.8, 23.7, 22.5. UV-vis (H₂O): λ_{\max} = 382 nm; PL (H₂O): λ_{\max} = 417 nm. ϵ = 4.56×10^4 M⁻¹cm⁻¹ per monomer unit.

5 **Example 11.** Cationic water-soluble polymer (M₂₅P₇₅⁺).

Condensed trimethylamine (2 mL) was added dropwise to a solution of the neutral polymer M₂₅P₇₅ (60 mg) in THF (10 mL) at -78 °C. The mixture was allowed to warm up to room temperature. The precipitate was re-dissolved by the addition of water (10 mL). After the mixture was cooled down to -78 °C, extra trimethylamine (2 mL) was added and
10 the mixture was stirred for 24 h at room temperature. After removing most of the solvent, acetone was added to precipitate M₂₅P₇₅⁺ (72 mg, 89%) as an off-white powder. ¹H NMR (500 MHz, CD₃OD): δ 8.0-7.5 (m, 10H), 3.3 (br, 4H), 3.1 (s, 18H), 2.3 (br, 4H), 1.6 (br, 4H), 1.2 (br, 8H), 0.9 (br, 4H). UV-vis (H₂O): λ_{\max} = 376 nm; PL (H₂O): λ_{\max} = 417 nm. ϵ = 4.22×10^4 M⁻¹cm⁻¹ per monomer unit.

15 **Example 12.** Cationic water-soluble polymer (M₅₀P₅₀⁺).

Condensed trimethylamine (2 mL) was added dropwise to a solution of the neutral polymer M₅₀P₅₀ (60 mg) in THF (10 mL) at -78 °C. The mixture was allowed to warm up to room temperature. The precipitate was re-dissolved by the addition of water (10 mL). After the mixture was cooled down to -78 °C, extra trimethylamine (2 mL) was added and
20 the mixture was stirred for 24 h at room temperature. After removing most of the solvent, acetone was added to precipitate M₅₀P₅₀⁺ (68 mg, 84%) as an off-white powder. ¹H NMR (500 MHz, CD₃OD): δ 8.1-7.7 (m, 10H), 3.3 (br, 4H), 3.1 (s, 18H), 2.3 (br, 4H), 1.6 (br, 4H), 1.3 (br, 8H), 0.8 (br, 4H). UV-vis (H₂O): λ_{\max} = 337 nm; PL (H₂O): λ_{\max} = 403 nm. ϵ = 3.15×10^4 M⁻¹cm⁻¹ per monomer unit.

25 **Example 13.** Cationic water-soluble polymer (M₇₅P₂₅⁺).

Condensed trimethylamine (2 mL) was added dropwise to a solution of the neutral polymer M₇₅P₂₅ (60 mg) in THF (10 mL) at -78 °C. The mixture was allowed to warm up to room temperature. The precipitate was re-dissolved by the addition of water (10 mL). After it was cooled down to -78 °C, extra trimethylamine (2 mL) was added and the

mixture was stirred for 24 h at room temperature. After removing most of the solvent, acetone was added to precipitate $M_{75}P_{25}^+$ (70 mg, 87%) as an off-white powder. 1H NMR (500 MHz, CD_3OD): δ 8.0-7.7 (m, 10H), 3.3 (br, 4H), 3.1 (s, 18H), 2.3 (br, 4H), 1.6 (br, 4H), 1.3 (br, 8H), 0.8 (br, 4H). UV-vis (H_2O): $\lambda_{max} = 347$ nm; PL (H_2O): $\lambda_{max} = 410$ nm. $\epsilon = 2.98 \times 10^4$ $M^{-1}cm^{-1}$ per monomer unit.

Example 14. Cationic water-soluble polymer ($M_{90}P_{10}^+$).

Condensed trimethylamine (2 mL) was added dropwise to a solution of the neutral polymer $M_{90}P_{10}$ (60 mg) in THF (10 mL) at -78 °C. The mixture was allowed to warm up to room temperature. The precipitate was re-dissolved by the addition of water (10 mL). After the mixture was cooled down to -78 °C, extra trimethylamine (2 mL) was added and the mixture was stirred for 24 h at room temperature. After removal most of the solvent, acetone was added to precipitate $M_{90}P_{10}^+$ (61 mg, 75%) as an off-white powder. 1H NMR (500 MHz, CD_3OD): δ 8.0-7.7 (m, 10H), 3.3 (br, 4H), 3.1 (s, 18H), 2.3 (br, 4H), 1.6 (br, 4H), 1.3 (br, 8H), 0.8 (br, 4H). UV-vis (H_2O): $\lambda_{max} = 362$ nm; PL (H_2O): $\lambda_{max} = 421$ nm. $\epsilon = 3.24 \times 10^4$ $M^{-1}cm^{-1}$ per monomer unit.

Example 15. The absorption spectra of the cationic polymers ($M_{100}P_0^+$, $M_{90}P_{10}^+$, $M_{75}P_{25}^+$, $M_{50}P_{50}^+$, $M_{25}P_{75}^+$, $M_0P_{100}^+$). There is a clear blue shift (from 380 nm for $M_0P_{100}^+$ to 337 nm for $M_{100}P_0^+$) with increasing fraction of *meta* linkages in the random copolymers. Such a spectral blue shift is expected on the basis of the less effective conjugation across the *meta* linkage along the polymer main chain. The representative absorption spectra are shown in Figure 1.

Example 16. The emission spectra of the cationic water-soluble polymers ($M_{100}P_0^+$, $M_{90}P_{10}^+$, $M_{75}P_{25}^+$, $M_{50}P_{50}^+$, $M_{25}P_{75}^+$, $M_0P_{100}^+$). There is a red-shift in the emission of the polymers with increasing fraction of *para* linkages. However, the maximum of the emission saturates more quickly, as compared to the absorption spectra. Efficient intramolecular energy transfer occurs, since introducing 10% *para* linkage shifts the polymer emission from 369 nm (all *meta* linkage) to 400 nm. Due to the efficient intramolecular energy transfer, polymers of 50% or higher *para* linkers have very similar emission spectra. Representative emission spectra are shown in Figure 2.

Example 17. The emission spectra of cationic water-soluble polymers $M_{50}P_{50}^{+}$ and $M_0P_{100}^{+}$ and the absorption spectrum of ds-DNA-C* (C* = fluorescein) (shown in Figure 3). The ds-DNA-C* was prepared by annealing the ss-DNA-C* probe (sequence 5'-C*-ATC TTG ACT ATG TGG GTG CT) at 2 °C below its melting point (59.5 °C) in the presence of an equimolar amount of its complementary 20 base pair (5'-AGC ACC CAC ATA GTC AAG AT) for 20 min. The data show that, despite their different molecular structures, there is nearly identical overlap between the emission of the polymers and the ds-DNA-C* absorption. The optical requirements for Förster energy transfer are equally met for both materials.

Example 18. Comparison of energy transfer between the cationic water-soluble polymers ($M_{100}P_0^{+}$, $M_{90}P_{10}^{+}$, $M_{75}P_{25}^{+}$, $M_{50}P_{50}^{+}$, $M_{25}P_{75}^{+}$, $M_0P_{100}^{+}$) and ds-DNA-C*. The ds-DNA-C* was prepared by annealing the ss-DNA-C* probe (sequence 5'-C*-ATC TTG ACT ATG TGG GTG CT) at 2 °C below the melting point (59.5 °C) in the presence of an equimolar amount of its complementary 20 base pair (5'-AGC ACC CAC ATA GTC AAG AT) for 20 minutes. Measurements were carried out in a buffered solution (50 mmol phosphate buffer pH = 8.0) and at a fixed ds-DNA-C* concentration (2.0 E-8 M). Figure 4 shows a direct comparison of the emission of C* upon excitation of the polymers in the presence of ds-DNA-C*. The excitation wavelength is the maximum absorption of each polymer. At a polymer concentration of 5.0 E-7 M in repeat units, the emission from ds-DNA-C* (upon excitation of the polymers) is higher for the random copolymers, as compared to the all *para*, rigid rod polymer. A schematic illustration of the difference in polymer/ds-DNA-C* complexation is shown in Figure 5.

Example 19. Figure 6 shows a direct comparison of the C* emission against polymer concentration in buffer upon excitation of $M_{50}P_{50}^{+}$ or $M_0P_{100}^{+}$. Measurements were carried out in buffer (50 mmol phosphate buffer pH = 8.0), at a fixed ds-DNA-C* concentration ($[ds-DNA-C*] = 2.0 \text{ E-8 M}$), with the polymer concentration varying from 1.0 E-7 M to 1.0 E-6 M. The excitation wavelength for both polymers was chosen at 363 nm, where the optical density for both polymers is nearly identical. Taking into account that an equal number of polymer excitations are generated, the C* emission is twice more

intense for $M_{50}P_{50}^{+}/ds\text{-DNA-C}^{*}$ than for $M_0P_{100}^{+}/ds\text{-DNA-C}^{*}$ at each polymer concentration.

Example 20. A scheme for the use of a cationic water-soluble conjugated polymer with a specific ss-DNA-C* optical probe to detect a complementary ss-DNA sequence is shown in Figure 7.² In a homogeneous assay, one excites the conjugated polymer and compares the emission of the dye (C*) attached to the probe ss-DNA (shown in red) to that of the conjugated polymer (shown in black). The optical properties of the components are chosen so that only the conjugated polyelectrolyte absorbs at the excitation frequency and the absorption of C* overlaps the emission from the conjugated polyelectrolyte. Hybridization of the ss-DNA-C* probe strand to its complementary strand (shown in blue) results in a more efficient FRET ratio (A) than when a non-complementary strand (shown in green) is present in the solution (B).

Example 21. Comparison of the emission of C* upon excitation of $M_{50}P_{50}^{+}$ or $M_0P_{100}^{+}$ in the presence of (i) ds-DNA-C* and (ii) ss-DNA-C*/ non-complementary ss-DNA (Figure 8). The DNA-C* probe with a specific sequence 5'-C*-ATC TTG ACT ATG TGG GTG CT) was annealed 2 °C below the melting point (59.5 °C) for 20 minutes in the presence of an equimolar amount of its complementary 20 base pair (5'-AGC ACC CAC ATA GTC AAG AT) and in an identical fashion with an non-complementary 20 base ss-DNA (5'-GAC TCA ATG GCG TTA GAC TG). Measurements were carried out in buffer (50 mmol phosphate buffer pH = 8.0) with $[ds\text{-DNA-C}^{*}] = [ss\text{-DNA-C}^{*}] = 2.0E\text{-}8$ M.

At a polymer repeat unit concentration of $4.2E\text{-}7$ M, one observes two important differences. First, the ds-DNA-C* emission is more intense for the $M_{50}P_{50}^{+}$ than $M_0P_{100}^{+}$; Second, the C* emission intensity ratio for ds-DNA-C*/(ss-DNA-C*+ss-DNA) is larger for $M_{50}P_{50}^{+}$. Therefore, the use $M_{50}P_{50}^{+}$ yields more intense signal and lower background emission than $M_0P_{100}^{+}$, which provides a DNA assay that is more discriminating.

Example 22. An example of using $M_{50}P_{50}^{+}$ as an energy transfer donor for a strand specific DNA assay which takes advantage of chromophore (C*) labeled peptide nucleic acid (PNA) probe strands. In PNAs, the negatively charged phosphate linkages in DNA are replaced by peptomimetic amide linkages. Consequently, non-specific electrostatic

interactions between cationic water-soluble polymers and the PNA-C* probe will be greatly reduced, as compared to the same situation where ss-DNA-C* is used as a probe.¹ Similar to the method described in Example 17 for annealing with DNA, the PNA-C* probe ([PNA-C*] = 2.0E-8 M, sequence 5'-C*-CAG TCC AGT GAT ACG-3') was
5 annealed 2 °C below its melting point (72 °C) in the presence of an equimolar amount of its complementary 15 base pair ss-DNA (5'-CGT ATC ACT GGA CTG-3') and in an identical fashion with a non-complementary 15 base ss-DNA (5'-ACT GAC GAT AGA CTG-3'). Measurements were carried out in buffer (50 mmol phosphate buffer pH = 6.0) and at a fixed PNA-C* concentration of 2.0E-8M. Figure 9 shows representative data for
10 this type of assay using the copolymer of M₅₀P₅₀⁺.

Example 23. UV-vis and fluorescence spectra for a range of compositions are summarized in Table 3. There is a progressive blue shift in absorption with increasing *meta* content, consistent with the more effective electronic delocalization across *para* linkages. The ϵ values are lowest for polymers with intermediate compositions because the
15 random distribution of conjugated segments results in broader absorption bands.

Table 3. Optical properties of the polymers.

M _n P _m ⁺	$\lambda_{\text{max, abs}}$	$\lambda_{\text{max, em}}$	ϵ^a	Φ_{buffer}^b
M ₁₀₀ P ₀ ⁺	335	369	37	0.51
M ₉₀ P ₁₀ ⁺	337	403	32	0.57
M ₇₅ P ₂₅ ⁺	347	410	30	0.50
M ₅₀ P ₅₀ ⁺	361	421	32	0.44
M ₂₅ P ₇₅ ⁺	376	417	42	0.42
M ₀ P ₁₀₀ ⁺	384	417	46	0.42

a. unit: 10³ Lcm⁻¹mol⁻¹

b. 50 mmol phosphate buffer, quinine bisulfite as the standard

Figure 2 shows the fluorescence spectra in water as a function of polymer
20 composition. Increasing the *para* content past the 50:50 ratio does not perturb the emission maxima. Fast energy transfer, either by intra- or interchain mechanisms, localizes excitations on the longest conjugation segments within the lifetime of the excited state.¹⁸

Table 3 shows that there is little variation in the fluorescence quantum yields (Φ in Table 3).

Equation 1 describes how the FRET rate changes as a function of the donor-acceptor distance (r), the orientation factor (κ), and the overlap integral (J).

$$k_t(r) \propto \frac{1}{r^6} \cdot \kappa^2 \cdot J(\lambda) \quad (1)$$

$$J(\lambda) = \int_0^{\infty} F_D(\lambda) \epsilon_A(\lambda) \lambda^4 d\lambda$$

5

Since $M_{50}P_{50}^+$ and $M_{100}P_{100}^+$ have similar emission frequencies, the value of J using a common acceptor dye should be nearly identical between the two polymers. The fluorescence lifetimes of the two polymers are similar (400 ± 50 ps). Therefore, differences in FRET efficiencies to a common acceptor chromophore can extract information relevant to the average polymer/acceptor chromophore distance and the orientation of transition moments.

To examine the effect of polymer structure on the interactions with a biological substrate, we examined FRET from $M_{50}P_{50}^+$ or $M_{100}P_{100}^+$ to a double stranded DNA containing fluorescein (C^*) at the 5' position (dsDNA- C^*). Figure 6 shows the C^* emission intensity as a function of polymer concentration, upon excitation of $M_{50}P_{50}^+$ or $M_{100}P_{100}^+$ at 363 nm. This wavelength was chosen because there is no significant C^* absorption and the two polymers have similar ϵ values. Excitation thus leads to a similar number of polymer-based excited states. We also confirmed that the value of Φ for C^* is the same in the two sets of solutions. The data in Figure 6 show more efficient FRET from $M_{50}P_{50}^+$, consistent with a shorter distance to dsDNA- C^* , and/or with more variable orientation of the transition moments (improved κ).

A second set of experiments involved FRET from the CCPs to ds-DNA with intercalated ethidium bromide (EB).^{19,20} EB emission occurs only from FRET to the intercalated moieties, upon excitation of the CCPs. Figure 10 shows more efficient transfer in the series $M_{100}P_{100}^+ \rightarrow M_{25}P_{75}^+ \rightarrow M_{50}P_{50}^+ \rightarrow M_{75}P_{25}^+$ ($M_{100}P_0^+$ was not tested because its

25

emission spectrum does not overlap significantly the absorption spectra of EB ($\lambda_{\text{max,abs}} = 530 \text{ nm}$). A clear improvement in FRET therefore takes place with increased *meta* contents in the polymer.

5

Although the invention has been described in some detail with reference to the preferred embodiments, those of skill in the art will realize, in light of the teachings herein, that certain changes and modifications can be made without departing from the spirit and
10 scope of the invention. Accordingly, the invention is limited only by the claims.

References

- ¹ Gaylord, B. S.; Heeger, A. J.; Bazan, G. C. DNA detection using water-soluble conjugated polymers and peptide nucleic acid probes. *Proc. Natl. Acad. Sci. USA.* 2002, 99, 10954.
- ² Gaylord, B. S.; Heeger, A. J.; Bazan, G. C. DNA hybridization detection with water-soluble conjugated polymers and chromophores labeled single stranded DNA. *J. Am. Chem. Soc.* 2003,
- ³ Bronich, T. K.; Nguyen, H. K.; Eisenberg, A.; Kabanov, A. V. Recognition of DNA topology in reactions between plasmid DNA and cationic copolymers. *J. Am. Chem. Soc.* 2000, 122, 8339.
- ⁴ Principles of fluorescence spectroscopy. (Lakowicz, J R. Ed.) Kluwer academic, Plenum Publishers, New York, 1999).
- ⁵ Chen, L.; McBranch, D. W.; Wang, H. L.; Helgeson, R.; Wudl, F.; Whitten, D. G. *Proc. Natl. Acad. Sci. U.S.A.* 2000, 96, 12287.
- ⁶ McQuade, D.T.; Pullen, A. E.; Swager, T.M. *Chem. Rev.* 2000, 100, 2537.
- ⁷ Pinto, M. R.; Schanze, K. S. *Synthesis-Stuttgart.* 2002, 9, 1293.
- ⁸ Baur, J. W.; Kim, S. H.; Balanda, P. B.; Reynolds, J. R.; Rubner, M. F. Thin-Film light-emitting devices based on sequentially adsorbed multilayers of water-soluble poly(p-phenylene)s. *Adv. Mater.* 1998, 10, 1452.
- ⁹ Nielsen, P. E.; Egholm, M. *Peptide Nucleic Acids: Protocols and Applications.* Horizon Scientific Press, Portland, 1999.
- ¹⁰ K. Nagai, I. W. Mattay, *RNA-Protein Interactions. Frontiers in Molecular Biology Series* (Oxford University Press, Oxford, UK, 1994).
- ¹¹ G. Varani, *Acc. Chem. Res.* 30, 189 (1997).
- ¹² Y. N. Vaishnav, F. Wong-Staal, *Ann. Rev. Biochem.* 60, 577 (1991).
- ¹³ C. Jain, J. G. Belasco, *Methods Enzymol.* 318, 309 (2000).
- ¹⁴ B. A. Sullenger, E. Gilboa, *Nature* 418, 252 (2002).
- ¹⁵ E. V. Pilipenko et al., *Genes Dev.* 14, 2028 (2000).
- ¹⁶ Portela, P. Digard, J. *Gen. Virol.* 83, 723 (2001).

-
- ¹⁷ G. T. Hermanson, *Bioconjugate Techniques*, Academic Press, San Diego, 1996.
- ¹⁸ Miao, Y. J.; Herkstroeter, W. G.; Sun, B. J.; Wong-Foy, A. G.; Bazan, G. C. *J. Am. Chem. Soc.* 1995, 117, 11407.
- ¹⁹ LePecq, J. B.; Paoletti, C. *J. Mol. Biol.* 1967, 27, 87.
- ²⁰ Morgan, A. R.; Pulleyblank, D. E. *Biochem. Biophys. Res. Commun.* 1974, 61, 346.